Quantifying root growth dynamics and nutrient uptake in apple trees

By

Frederik Jacobus van Zyl

Thesis presented in fulfilment of the requirements for the degree of Master of Science in the Faculty of Agrisciences at Stellenbosch University

Supervisor:	Dr E. Lötze	Dept. of Horticultural Science Stellenbosch University
Co-supervisor:	Dr N.J. Taylor	Dept. of Plant and Soil Science University of Pretoria
Co-supervisor:	Dr J.C. Fourie	ARC Infruitec-Nietvoorbij Stellenbosch

March 2016

DECLARATION

I, the undersigned, hereby declare that the entirety of the work contained in this thesis is my own original work and that I have not previously, in its entirety or in part, submitted it at any university for obtaining any qualification.

Signature Date

…… ……

SUMMARY

The dynamics of white roots were quantified using minirhizotrons (MR) over two consecutive seasons in four apple orchards in the Elgin-Vyeboom region of the Western Cape, South Africa. The cultivars monitored in this study were as follows: young, non-bearing 'Corder Gala'/M7; young, bearing 'Fuji'/M793; mature, bearing 'Golden Delicious'/M793 and 'Cripps Pink'/M793. Root growth patterns were related to soil water and temperature dynamics to determine the influence of the soil environment on white root dynamics. Changes in photosynthesis for the non-bearing 'Corder Gala' and bearing 'Golden Delicious' orchards were also quantified in order to determine possible correlation between white root activity and tree physiology.

Nutrient uptake and distribution in relation to white root dynamics and established uptake periods were also investigated for one-year-old potted 'Golden Delicious'/M7 trees (glasshouse) and for mature bearing 'Golden delicious'/M793 (field). In the potted trial, the effect of timing and application rate of soil applied $Ca(NO_3)_2$ was evaluated with reference to Ca concentration and distribution amongst the roots, trunk and new growth. In the field trial, the effect on fruit tree performance was evaluated following the soil application of $Ca(NO_3)_2$ during white root flushes (determined by MR images) compared to recommended phenological based timmings (90 % petal drop and post-harvest).

A bimodal white root growth pattern was confirmed for the bearing orchards with the first root flush in summer and a second, often longer flush, in winter. The winter root flush during tree dormancy is unique compared to existing literature on apple root growth dynamics and is attributed to the warmer winter climate of our region. For the non-bearing orchard, no defined white root growth pattern was observed. It appears that white root production occurs throughout the growing season in varying quantities from spring until autumn.

White root growth dynamics in this study was not determined by the seasonal variation in soil temperature, although soil environmental conditions did play a role. The consistent white root growth flush for bearing orchards during winter indicate suitable environmental soil conditions for root growth - which is in contrast to climatic regions where winter temperatures result in cold (<5°C) soils supressing root growth. Similarly, soil water fluctuation did not appear to influence the timing of the flushes, especially for the bearing 'Golden Delicious' orchard. Soil

water and temperature in this study was therefore condusive to root growth throughout the year. This suggests that other factors e.g. endogenous tree physiological factors probably control the temporal pattern of white root production in these orchards. Changes in white root numbers and photosynthesis from spring to autumn indicated a possible relationship in the non-bearing orchard, but was not evident in the mature bearing orchard.

In the potted trial, both the timing and concentration of soil applied $Ca(NO_3)_2$ affected Ca distribution in roots and new growth. The effect of application time significantly influenced the Ca concentration of both the roots and new growth and confirmed previous findings, whereas the effect of application rate only influenced the Ca concentration of the new aerial growth. Higher rates of $Ca(NO_3)_2$ in summer was neccesary to significantly increase the Ca concentration of new arial growth, whereas the standard rate was suffice in autumn for significant increases in the root system. In the field trial however, no significant affect of additional $Ca(NO_3)_2$ for the applied rates was observed as quantified by leaf and fruit mineral analysis or fruit yield and quality.

OPSOMMING

Wit wortel dinamika is gemonitor in vier appelboorde en gekwantifiseer met behulp van minirhizotrons (MR) oor twee seisoene in die Elgin-Vyeboom streek van die Wes-Kaap, Suid-Afrika. Die kultivars in die studie was soos volg: jong nie-draende ‘Corder Gala’/M7; jong, draende ‘Fuji’/M7; volwasse, draende ‘Golden Delicious’/M793 en ‘Cripps Pink’/M793. Wortel groeipatrone is vergelyk met grondwater en -temperatuur dinamika om die invloed van die grondomgewing op wit wortel dinamika te bepaal. Verandering in fotosintese vir die jong nie-draende ‘Corder Gala’/M7 en die volwasse, draende ‘Golden Delicious’/M793 boorde is ook gekwantifiseer om ‘n moontlike verwantskap te ondersoek tussen witwortel aktiwiteit en boom fisiologie. Ca opname en weefsel verspreiding ten opsigte van witwortel dinamika en bepaalde tydperke van Ca opname is ook bepaal vir een-jaar-oue ‘Golden Delicious’/M7 bome in potte onder glashuis toestande en vir volwasse, draende ‘Golden Delicious’/M793 onder veldtoestande. In die potproef is die effek van tydsberekening (somer, herfs sowel as somer en herfs) en toedieningsdosis (1X of 3X) van $Ca(NO_3)_2$ grondtoedienings ge-evalueer ten opsigte van Ca-konsentrasie en -weefselverspreiding tussen die wortels, stam en nuwe groei (lote en blare). In die veldproef, is die effek van additionale $Ca(NO_3)_2$ grondtoedienings tydens die aktiewe witwortel groei periodes (bepaal deur MR data) teenoor die aanbevele fenologies

gebasseerde toedienings tye (90 % blomblaarval en na-oes), sowel as n kontrole (geen additionele $Ca(NO_3)_2$), vergelyk ten opsigte van boom prestasie.

‘n Bi-modale wortel groeipatroon is bevestig vir die draende boorde, met die eerste witwortel groei fase in somer en die tweede, dikwels groter groei fase, in die winter. Die winter wortel groeifase gedurende dormansie is egter uniek. Vir die nie-draende boord is geen duidelike patroon opgemerk nie. Dit het egter voorgekom asof witwortel produksie regdeur die seisoen vanaf lente tot herfs plaasvind, alhoewel dit in wisselende hoeveelhede gedurende hierdie tydperk voorkom. Witwortel groei tendense het dus verskil tussen draende en nie-draende bome.

Witwortel dinamika in hierdie studie was nie gekorreleer aan die seisoenale verandering in grondtemperatuur nie, alhoewel grondtemperatuur wel ‘n rol speel. Die konstante witwortel groeifase gedurende die winter in die draende boorde in hierdie studie, dui op geskikte omgewingstoestande vir wortelgroei gedurende die winter wat in kontras is met ander klimaatstreke waar koue (<5°C) grondtemperature wortelgroei in die winter onderdruk. Soortgelyk blyk dit dat grondwater ook nie die aanvang en duur van ‘n wortel groeifase beheer nie, veral nie vir die draende ‘Golden Delicious’ boord nie. Grondwater en -temperatuur in hierdie studie was dus gunstig vir wortelgroei regdeur die jaar. Dit is dus moontlik dat ander faktore bv. interne boom fisiologiese faktore die tydsberekening van die wortel groeifases in hierdie boorde bepaal in die Elgin-Vyeboom area. Veranderinge in witwortel getalle en fotosintese gedurende die lente tot herfs is ‘n aanduiding van ‘n moontlike verwantskap in die nie-draende boord, maar dit is nie waargeneem in die draende boord nie.

In die pot proef het beide die tydsberekening sowel as die konsentrasie van $Ca(NO_3)_2$ toediening ‘n betekenisvolle effek op die verspreiding van Ca in die wortels en nuwe groei gehad. Die effek van tyd van toediening het die Ca konsentrasie van die wortels en nuwe groei noemenswaardig beïnvloed in ooreenstemming met bestaande literatuur, terwyl die effek van toedieningsdosis net die Ca konsentrasie van die nuwe groei betekenisvol beïnvloed het. ‘n Hoër $Ca(NO_3)_2$ dosis in die somer was nodig om die Ca konsentrasie van die nuwe groei noemenswaardig te laat toeneem, terwyl die standard dosis in die herfs voldoende was om die Ca konsentrasie van die wortels te verhoog. Daarinteen is geen noemenswaardige invloed

opgemerk met betrekking tot die effek van $Ca(NO_3)_2$ in reaksie op die aanbevole dosis op die Ca-konsentrasie van blare en vrugte, sowel as opbrengs en vrugkwaliteit in die veldproef nie.

DEDICATION

Dedicated to my family

ACKNOWLEDGEMENTS

I am gratefully indebted to the following institutions and people:

University of Stellenbosch for promoting high quality education

My supervisor, Dr E. Lötze, for her time, encouragement, insights and assistance in the field

My co-supervisor, Dr N.J. Taylor, for her time, excellent critique and contributions

Mr G. Lötze and his staff (esspecially Mr A. Swartz) in the Department of Horticulture Science, University of Stellenbosch for all their assistance in the field and laboratory

Hortgro Science and Yara Western Cape for funding this project

Applegarth (Roux Niewoudt) for accommodating the trial on the farm and assistance from farm labour during harvest

Vyeboom Farms (Crooks Brothers) for accommodating the young, non-bearing trial

My family and friends for their love and support

TABLE OF CONTENTS

This thesis presents a consolidation of manuscripts where each paper is an individual entity. Some repetition between chapters has therefore been unavoidable.

General Introduction

The temporal growth pattern of fine roots may have important implications for nutrient uptake and carbon partitioning in fruit trees (Eissenstat et al., 2006). Most of the earlier and current reports on apple root growth patterns originate from temperate climates of the Northern hemisphere. Very few reports on fine root dynamics of fruit trees exist for warmer climates of the Southern hemisphere (Cripps, 1970). Existing reports on apple root growth patterns show substantial variation with regard to timing and duration of fine root production (Atkinson and Wilson, 1980; Psarras et al., 2000; Ma et al., 2013). Contributing factors that influence the temporal production of fine roots in fruit orchards include climate, rootstock-scion combination, soil type, soil water and -temperature dynamics, as well as cultural practices such as prunning, cropping intensity, irrigation and fertilization - which in turn affect the carbon balance of the tree (Atkinson and Wilson, 1980; Eissenstat et al., 2006; Ma et al., 2013; Rogers and Head, 1969; Yao et al., 2009). The complex interaction between these physiological and environmental factors, especially in bearing trees, increases the difficulty in accurately predicting the timing of root growth flushes for a particular orchard. Factors controlling root growth patterns of woody perennials are substantially more complex compared to annual plants due to their perennial habit, large size and ability to store resources and may therefore vary greatly between species and climates (Rogers and Head, 1969). Minirhizotrons were used in this study as a non-destructive method for monitoring and recording the emergence, development and senescence of roots over time (Fukuzawa et al., 2012; Gluszek et al., 2013; Vamerali et al., 2012; Withington, 2005).

Under temperate climatic conditions, various root growth patterns have been reported for apple. Many early studies reported bimodal (two main seasonal peaks) patterns for root production, with the first peak between full bloom and late spring - before the main shoot growth phase, while the second peak usually occurs after shoot growth rates declined, or following harvest (Atkinson and Wilson, 1980; Cripps, 1970; Fallahi, 1994; Head, 1967; Rom, 1996). Timing of root growth in this bimodal pattern seems to alternate with phenological events, suggesting competition between sinks for assimilates. More recent studies show the predictability of apple root growth dynamics to be more complex than what the bimodal theory suggests. Psarras et al. (2000) observed a single peak in root production which overlapped with high shoot and fruit growth rates for two consecutive seasons. The consistency of temporally similar root growth

peaks between seasons also varied, as the occurrance of a spring or autumn root flush were reported to alternate between years (Eissenstat et al., 2006).

A root growth flush is characterized by the production of white roots within a relatively concentrated phase of activity which occurs periodically throughout the season, especially for bearing fruit trees or where seasonal environmental conditions limit root growth (Eissenstat et al., 2006; Kuhns et al., 1985; Montagnoli et al., 2014; Psarras et al., 2000; Yao et al., 2006). White roots are short lived, primary roots that originate from older or more mature roots and are thought to specialize in nutrient uptake and cytokinin synthesis (Baldi et al., 2010; Ma et al., 2013). Morphological and physiological changes are associated with root maturation - as a white root decreases in diameter and turns brown due to cortex senesence and tannin deposition. The root cortex is associated with high metabolic activity and nutrient uptake potential which consequently decreases following root browning. During this transition from white to brown, the casparian band becomes fully developed through lignin and suberin depostion in the endodermal cell layer interupting continuous apoplastic pathways from the root surface into the stele further decreasing the nutrient uptake potential of the root (Nightingale, 1935, White, 2001). A white root growth flush may therefore signify a window of opportunity for maximizing uptake of soil applied fertilizer especially for apopastically transported elements such as Ca (Baldi et al., 2010; Ferguson, 1980; Marschner, 1995; White, 2001).

The aim of this thesis was to quantify the dynamics of white root growth for young and mature apple trees in the Western Cape region of South Africa. In addition, we aimed at determining the predominant factors that control the onset and duration of white root growth flushes within the climatic conditions of the Elgin-Vyeboom region. White root growth patterns were related to environmental factors (soil water and -temperature dynamics) as well as tree physiology (photosynthesis) in order to determine possible relationships. In addition, the effect of synchronizing soil $Ca(NO_3)_2$ applications with white root growth flushes and established uptake periods on Ca uptake was also evaluated through destructive tissue analysis (young, potted trees) as well as leaf and fruit analysis (mature bearing trees).

References

Atkinson, D. and Wilson, S.A. 1980. The growth and distribution of fruit tree roots: some consequences for nutrient uptake. (eds.) Atkinson, D., Jackson, J. E., Sharples, R. O. and Waller, W. M. *Mineral nutrition of fruit trees*, Butterworths publishers, 1980, 137-150.

Baldi, E., Wells, C. E. and Marangoni, B. 2010. Nitrogen absorption and respiration in white and brown peach roots. Journal of Plant Nutrition 33, 461-469.

Cripps, J. E. L. 1970. A seasonal pattern of apple root growth in Australia. Journal of Horticultural Science 45, 153-161.

Eissenstat, D. M., Lakso, A. N. Neilsen, D., Neilsen, G. H. and Smart, D. R. 2006. Seasonal patterns of root growth in relation to shoot phenology in Grape and Apple. Acta Horticulturae 721, 21 -26.

Fallahi, E. 1994. Root physiology, development and mineral uptake. p.19-30. In: A.B. Peterson and R.G. Stevens (eds.), Tree Fruit Nutrition: A Comprehensive Manual of Deciduous Tree Fruit Needs. Good Fruit Grower, Yakima, Washington, USA.

Ferguson, I. B. 1980. Uptake and transport of calcium. Mineral nutrition of fruit trees (eds.) Atkinson, D., Jackson, J. E., Sharples, R. O. and Waller, W. M. Butterworths publishers, 1980, 183 – 192.

Fukuzawa, K., Dannoura, M. and Shibata, H. 2012. Fine root dynamics and root respiration. Measuring roots, Mancuso, S (ed.), Springer- Verlag Berlin Heidelberg 2012, Chapter 15, p. 341-356.

Gluszek, S., Paszt, L. S., Sumorok, B., Derkowska, E. and Kozera, R. 2013. Application of the minirhizotron technique to studying the roots of fruit plants. Advances in Science and Technology Research Journal 7(18), 45–53.

Head, G.C. 1967. Effects of seasonal changes in shoot growth on the amount of unsuberized root on apple and plum trees. J. Hort. Sci. 42:169-180.

Kuhns, M. R., Garrett, H. E., Teskey, R. O., and Hinckley, T. M. 1985. Root growth of black walnut trees related to soil temperature, soil water potential, and leaf water potential. Forest Science, 31(3), 617-629.

Ma, L., Hou, C. W., Zhang, X. Z., Li, H. L., Han, De G., Wang, Y. and Han, Z. H. 2013. Seasonal growth and spatial distribution of Apple tree roots on different rootstocks or interstems. Journal of American Society of Horticultural Science 138(2), 79–87.

Marchner, H. 1995. Mineral nutrition of higher plants second edition. London. Academic press, 63-70.

Montagnoli, A., Di Iorio, A., Terzaghi, M., Trupiano, D., Scippa, G. S., and Chiatante, D. 2014. Influence of soil temperature and water content on fine-root seasonal growth of European beech natural forest in Southern Alps, Italy. European Journal of Forest research, 133(5), 957-968.

Nightingale, G. T. 1935. Effects of temperature on growth, anatomy, and metabolism of apple and peach roots. Botanical Gazette, 96 (4), 581-639.

Psarras, G., Merwin, I. A., Lakso, A. N. and Ray, J. A. 2000. Root growth phenology, root longevity, and rhizosphere respiration of field grown ‘Mutsu' Apple trees on ‘Malling 9' rootstock. Journal of the American Society for Horticultural Science, 125(5), 596-602.

Rogers, W.S. and G.C. Head. 1969. Factors affecting the distribution and growth of roots of perennial woody species, p. 111–148. In: W.J. Whittington (ed.). Root growth. Butterworths, United Kingdom.

Rom, C.R. 1996. Coordination of root and shoot growth: roots and rootstocks. p. 53-67. In: K.M. Maib, P.K. Andrews, G.A. Lang and K. Mullinix (eds.), Tree Fruit Physiology: Growth and Development. Good Fruit Grower, Yakima, Washington, USA.

Vamerali, T., Bandiera, M. and Mosca, G. 2012. Minirhizotrons in modern root studies. Measuring roots, Mancuso, S (ed.), Springer- Verlag Berlin Heidelberg 2012, 341-356.

White, P. J. 2001. The pathways of calcium movement to the xylem. Journal of Experimental Botany 52(358), 891-899.

Withington, J. M. 2005. Ph. D. Thesis in Ecology. Fine root production and lifespan in eleven temperate tree species growing in a common garden in Poland. Pennsylvania State University.

Yao, S., Merwin, I. A. and Brown, M. G. 2006. Root dynamics of apple rootstocks in a replanted orchard. HortScience, 41(5), 1149-1155.

Yao, S., Merwin, I. A. and Brown, M. G. 2009. Apple root growth, turnover, and distribution under different orchard ground cover management systems. HortScience, 44(1), 168-175.

Literature review

Factors affecting fine root growth dynamics and the relationship with nutrient uptake and tree physiology

Introduction

This literature review focusses on apple root growth dynamics and its role in nutrient uptake efficiency, as well as factors affecting the temporal and developmental aspects of fine root growth. Roots less than 2 mm in diameter are classified as fine roots (Ma et al., 2013; Terblanche, 1972; Yao et al., 2009) and fine root growth is typically used to determine the dynamics of root production and loss, i.e. root turnover (Ma et al., 2013). Newly produced fine roots of woody perennials are generally ephemeral (Wells and Eissenstat, 2003; Withington, 2005) and consume a significant portion of annually fixed carbon (C) assimilates due to their high turnover rates (Kozlowski, 1992). Various morphological and physiological changes occur during the development of a root (Taiz and Zeiger, 2010). Actively growing white roots have the greatest capacity for water and nutrient uptake because of the morphological and physiological root properties associated with this phase of root development (Baldi et al., 2010; Ma et al. 2013; Marschner, 1995; White, 2001). It is therefore important to know when root growth phases occur during the season in order to ensure an optimum nutrition strategy for fruit production (Atkinson and Wilson, 1980; Eissenstat et al., 2006). The dynamic presence of white roots is influenced by whole-tree physiology, as well as environmental conditions (Côté et al., 1998; Tierney et al., 2003). As both endogenous and environmental factors play a role in active root growth, it is difficult to predict the exact timing of root growth during the season. In contrast, the timing of bloom, shoot growth and fruit growth in deciduous trees is relatively well defined and confined to specific dates in spite of the influence of the same endogenous and environmental factors. The growth of the root system, however, is not confined to a particular pattern or season, as it does not become inherently dormant during unfavourable environmental conditions (Kozlowski et al., 1991).

Despite the huge variation between root systems of different plant species, these systems are generally highly adaptable (Green and Clothier, 1999; McManus and Veit, 2002). The ability of the root system to adapt is required to overcome the spatial differences in nutrients, water

and physical objects within the soil (Taiz and Zeiger, 2010). Besides physical and physiological adaptation, woody perinials also show temporal adaptation of fine root growth patterns (Eissenstat et al., 2006; Fukuzawa et al., 2012; Rogers and Head, 1969; Yao et al., 2006). Adaptability is thus also required to maintain a physiological balance between roots, shoots and reproductive growth in terms of photosynthate partitioning, nutrient demand and water requirements (Gregory, 2008). The timing of root growth in apple trees is therefore related to the C balance of the tree and has direct implications for nutrient uptake (Eissenstat et al., 2006).

Various factors, such as cultural practices, soil water availability, soil temperature, soil type, climate, carbon balance of the tree and root stock-scion combination influence the onset and duration of root growth cycles (Atkinson and Wilson, 1980; Eissenstat et al., 2006; Gregory, 2008; Kozlowski, 1992; Ma et al. 2013). A better understanding of these factors may help to explain the timing of the most active root growth flushes. Furthermore, a better understanding of root activity dynamics is a prerequisite for enhancing the uptake efficiency of a nutrient, such as Ca. The absorption of Ca is particularly sensitive to the developmental stage of the root tissue (Marschner, 1995; White, 2001), with the potential for Ca uptake being highest in younger root tissue where endodermis and suberin deposition is least developed (Marschner, 1995; White, 1998; 2001). Knowledge of root respiration and growth cycles can therefore be used to determine the highest potential for nutrient uptake (Psarras et al., 2000). Although root functionality in general is not exclusively associated with the most active root growth periods, functional differences do exist between root tissues in different stages of development, as well as between different roots of similar age and size in terms of potential nutrient uptake and water conductance (Green and Clothier, 1999; Taiz and Zeiger, 2010; Wells and Eissenstat, 2001).

Root growth cycles

Active growth of fine roots in woody perenials is not continuous throughout the growing season, especially in the case of bearing fruit trees (Eissenstat et al., 2006; Yao et al., 2006), or in natural forests, where environmental conditions are periodically limiting (Kuhns et al., 1985; Montagnoli et al., 2014). According to Ma et al. (2013), one to three distinct root growth peaks per year have been observed in apple trees. However, it is more common for seedlings or young establishing trees to have two or more flushes per annum (Wittington, 2005). For deciduous forests, root growth can occur throughout the year, although root growth fluctuated according to soil conditions (Burke and Raynal, 1994; Kuhns et al., 1985; Tierney et al., 2003), ceasing

in winter due to low temperatures and suppressed by low soil water availability in summer (Kuhns et al., 1985). The seasonal root growth pattern of many forest species also correspond to canopy development, with significant root production in spring when the canopy is increasing and mortality in autumn during leaf senescence (Burke and Raynal, 1994; Côté et al., 1998; Pregitzer et al., 2000). However, in commercial fruit tree orchards many other factors come into play, such as irrigation, fertilization, pruning and crop load, the degree of which significantly influences whole tree carbon balance (Rogers and Head, 1969; Yao et al., 2009). Assimilate partitioning and availability, as well as sink competition, comprise the endogenous component influencing root growth patterns in apple. Cultural practices, such as pruning, thinning and irrigation, may also affect certain aspects of the growth phases indirectly through affecting the carbon balance of the tree (Atkinson and Wilson, 1980; Fumey et al., 2011; Naschitz et al., 2010; Wang and Stutte, 1992). The primary soil environmental parameters that may affect root growth cycles include both soil water and temperature, which become particularly important when they are limiting to growth (Côté et al., 1998; Pregitzer et al., 2000). These parameters can also affect both the qualitative and quantitative aspects of the newly produced roots. Aspects such as the number of roots produced, as well as root diameter, turnover rate, cortex longevity and in some cases even the onset of the root growth cycle may be postponed when soil conditions are not suitable (Eissenstat et al., 2000; Kuhns et al., 1985). Soil water and temperature, however, do not seem to be the major parameters controlling fine root growth patterns (Côté et al., 1998; Gregory, 2008; Joslin et al., 2001). Even under suitable soil water and temperature conditions during summer or winter, root growth can be absent and is attributed to the lack of carbohydrates, due to the fruit sink in summer (Yao et al., 2006) or the absence of leaves during dormancy (Cripps, 1970).

Root growth seems to be governed by a set of genetically determined developmental rules, which are modulated through interaction with the environment, as well as endogenous physiological conditions (Atkinson and Wilson, 1980; Hodge et al., 2009; Osmont et al., 2007). Root growth dynamics for genetically identical trees may therefore vary according to different orchard micro climates and growth dynamics and can also differ between years for the same trees (Atkinson and Wilson, 1980; Yao et al., 2009). Under the same environmental conditions, root growth dynamics also differ according to rootstock variety (Ma et al., 2013) or species (Withington, 2005).

Apple root growth dynamics

Many of the early studies on apple root growth patterns reported a bimodal cycle i.e. two main root growth peaks per year (Atkinson and Wilson, 1980; Cripps, 1970; Fallahi, 1994; Head, 1967; Rom, 1996). The first root growth phase either peaked around full bloom (Fallahi, 1994; Rom, 1996) or late spring (Atkinson and Wilson, 1980; Cripps, 1970; Head, 1967) before the main shoot growth phase, whilst the second growth cycle commenced only after the rate of shoot growth declined or after harvest, indicating some competition between sinks for assimilates (Atkinson and Wilson, 1980; Maggs, 1963; Palmer, 1992). This bimodal root growth theory has recently come under debate following more contemporary research (Eissenstat et al., 2006; Li et al., 2003; Ma et al., 2013; Psarras et al., 2000; Yao et al., 2006; 2009) where the predictability of root growth patterns were found to be more complex. Annual root growth patterns of apple trees may range widely in terms of dynamics (Ma et al., 2013) and may occur during times of high canopy demand for resources, such as during high fruit growth rates (Psarras et al., 2000), which is in contrast to what was previously observed. With the bimodal theory, there seemed to be a balance between resource allocation and various growth processes, so that new root growth flushes occur at different times than that of shoot and fruit growth, which are dominant sinks (Flore and Layne, 1999; Heim et al., 1979; Palmer, 1992).

New techniques, e.g. minirhizotron (MR) tubes, allow non-destructive root monitoring throughout the year as part of a replicated experimental design (Eissenstat et al., 2006; Gluszek et al., 2013). Using MR, Eissenstat et al. (2006) showed a different root growth pattern to the bimodal model under orchard conditions for ‘Gala’/M9. These authors reported a strong root flush during bloom, followed by modest but continuous root growth throughout the remainder of the season, with no root flush after harvest in the first year. The following spring, no indication of root activity was observed during bloom, instead, root growth increased steadily during summer in the second year, declined near harvest time and was followed by a strong root flush after harvest. For ‘Golden Delicious’/M9, Eissenstat et al. (2006) reported no autumn root flush for two consecutive years, with only a flush around full bloom. Psarras et al. (2000) reported one main peak of root growth for ‘Mutsu’/M9, which partially coincided with periods of shoot growth and fruit growth for two consecutive years. Similar results were later reported by Yao et al. (2006), where root growth peaked between late May and July (November to January, Southern hemisphere), coinciding with the main phase of shoot and fruitlet growth (Bergh, 1990; Miqueloto et al., 2014). These studies suggest that sink competition, as a factor

controlling root growth, varies in magnitude between orchards due to the combined differences of genotype, environmental conditions and cultural practices (Psarras et al., 2000). These recent MR based findings reveal a more complex behaviour of apple root growth than the more historical accounts. MR technology offers a greater potential for replication than the more expensive, static rhizotron chambers and a potential for more continuous undisturbed observations than destructive sampling techniques used by earlier researchers (Eissenstat et al., 2006; Gluszek et al., 2013).

Psarras et al. (2000) ascribed the absence of root growth in early spring solely to water saturated soil conditions (caused by a fragipan at 40 cm soil depth), as the soil temperatures (15 °C) were adequate for root growth. Therefore, the effect of waterlogged soil conditions and its consequent effect on soil temperature is most likely responsible for the absence of root growth during winter and early spring in many cases (Eissenstat et al., 2006; Psarras et al., 2000). Hypoxic, or sometimes anoxic conditions, resulting from waterlogged soil conditions are detrimental to root growth due to oxygen being essential for root respiration (Comas et al., 2002; Gregory, 2008).

During summer, differences in crop load can be a major influence on root growth patterns in apple (Rogers and Head, 1969; Yao et al., 2009), as the fruit sink influences whole tree carbon partitioning and reduces total fine root production (Cripps, 1970; Flore and Layne, 1999; Maggs, 1963; Palmer, 1992). In a year with a high crop load, the dynamics of root growth was found to be more uniform over time, with lower total root counts than the previous year with a light crop, where root activity fluctuated and had more pronounced growth peaks (Yao et al., 2009). Two pronounced peaks were observed in the light crop year around late July and late August (Northern hemisphere) after heavy rains, which followed hot dry conditions. Besides the distinct differences in the pattern of root growth for the same trees for two consecutive years, root activity occurred intermittently throughout the season reflecting the complexity of parameters affecting root activity (Yao et al., 2009).

In general, the cessation of deciduous tree root growth during winter is ascribed to low soil temperatures (Côté et al., 1998; Kuhns et al., 1985; Pregitzer et al., 2000; Psarras et al., 2000). However, the root system itself does not seem to enter an endogenously controlled dormant state, and may use reserves for growth during a leafless state when soil conditions are favourable (Kozlowski et al., 1991). Active root growth has been observed in winter, but only

for the first season after planting, and has been attributed to the stimulation caused by root pruning and relatively mild winter temperatures (minimum soil temperature of 8.9 °C) of Western Australia (Cripps, 1970).

Environmental factors affecting root growth

Soil Temperature

Both root growth and development are temperature dependent processes (Gregory, 2008). Soil temperature influences plant root systems by determining the potential rate of expansion and development through affecting the metabolic rate, as well as root function (Fageria, 2013). This can be largely attributed to the fact that cells divide more rapidly at optimal temperatures than at lower or extreme temperatures. Lower temperatures tend to promote the development of whiter and thicker, but less branched roots than higher temperatures (Fageria, 2013; Nightingale, 1935). Water and nutrient uptake rate, which affect root growth, are negatively affected at lower temperatures due to decreased root respiration rates (Fageria, 2013; Gregory, 2008; Psarras et al., 2000). However, the processes involved in nutrient acquisition are able to acclimate to low temperatures following prolonged exposure (Gregory, 2008). The acclimatization response involves changes in lipid composition, the activity of membrane carriers, as well as the morphology and size of the root system. These responses have the overall effect of reducing the temperature dependence of ion transport, allowing adequate nutrient uptake at lower temperatures (Gregory, 2008). Soil temperature also affects oxygen consumption by roots and rhizosphere microorganisms, where consumption increases at higher temperatures (Fageria, 2013). Root growth usually increases with temperature until the optimal condition is reached, after which the rate of growth decreases. Optimal temperatures for root growth mainly depend on the plant species, but typically range between 25 and 35 °C according to Gregory (2008) and between 20 and 30 °C according to Fageria (2013). The minimum temperatures are between 0-12 °C, whilst maximum temperatures range between 40-45 °C respectively (Gregory, 2008). The minimum temperature threshold for apple root growth lies between 6°C (Rogers, 1939) and 8°C (Nightingale, 1935). Root dependent respiration for apple was found to be negligible below 5°C (Psarras et al., 2000). The upper temperature threshold for apple root initiation probably lies between 32 and 35°C as no new roots were produced at 35°C and very few at 32°C for young ‘Delicious’ trees in sand culture (Nightingale, 1935).

Factors affecting soil temperatures include: air temperature, intensity and duration of solar radiation, precipitation, evaporation, thermal conductivity and the type of soil surface management practice (Fageria, 2013). For example, ground cover management practices may significantly affect root mortality rates and therefore root distribution, mainly due to changes in shallow soil temperatures (Yao et al., 2009) - as different ground covers affect evaporation and radiation intensity at the soil surface. Air temperature may also have an effect on root growth, as higher temperatures increase the rate of leaf expansion and therefore light interception, increasing the available photosynthates for root growth (Gregory, 2008).

Temperatures in the root system environment also have implications for tree growth and phenology (Greer et al., 2005), which in turn may affect root growth cycles (Atkinson and Wilson, 1980; Rogers and Head, 1969). Shoot growth has been found to decrease at lower soil temperatures in apple (Greer et al., 2005). The timing and proportion of bud break, floral opening and shoot growth was significantly enhanced with increasing root zone temperatures (Greer et al., 2005). Furthermore, low soil temperatures are associated with more negative leaf water potentials, hence water stress is observed in newly planted trees due to low soil temperatures (Nambiar, 1983). Wilting may therefore be a consequence of low root temperatures due to decreased transmembrane transport (Marschner, 1995).

Nightingale (1935) showed that small differences in root temperature have extensive effects on root mass and anatomy of young apple and peach trees, as decreasing or increasing soil temperatures below or above 18 °C increased root volume significantly. Even a 3 °C difference in root temperature resulted in distinguishable differences in root growth. Besides its effect on the quantitative aspects of root growth, temperature also affects the qualitative properties of roots. Roots produced at temperatures below 24°C were more succulent, white and “typically of relatively large diameter” (Fageria, 2013; Nightingale, 1935). Roots developing at 24°C and higher were brown and lacked the same succulence, except near the tips (Nightingale, 1935). Nightingale (1935) also reported that, at temperatures lower than 18 °C, roots remained white for weeks, indicating that lower temperatures promote the longevity of juvenile cortical tissue, which is important for nutrient acquisition (White, 2001). Soil temperature also influences root turnover and longevity (Eissenstat et al., 2000; Gregory, 2008), as root respiration increases at higher temperatures, resulting in a shorter root life span, increasing root mortality rates (Eissenstat et al., 2000). This supports findings of Yao et al. (2009) where hot weather conditions (20 days > 32°C) decreased overall root growth, in addition to an increase in root

mortality rates, even though irrigation was supplied. Soil temperatures therefore increase carbon partitioning to roots because of the increased turnover rates (Eissenstat et al., 2000) and may also influence nutrient uptake due to increased rates of suberization under high temperatures (Kuhns et al., 1985; Nightingale, 1935; White and Broadly, 2003). Roots also seem to be very sensitive to the degree of short term soil temperature fluctuation (Fageria, 2013), often causing the top soil layer to be inhospitable to root growth (Nielsen, 1974; Pregitzer et al., 2000).

Soil water

Soil water plays a pivotal role with regards to root growth. It is the chemical and physical basis on which plant cells depend (Taiz and Zeiger, 2010). Water uptake rates of tree roots are highly influenced by soil water potential, as well as the water status of the tree (Green and Clothier, 1999). Insufficient soil water causes root cells to loose turgor to the detriment of cell expansion in the elongation zone, which is responsible for root growth (Taiz and Zeiger, 2010). Root diameter can decrease (Gregory, 2008; Sharp et al., 2004) and cell walls may change, becoming less permeable (to prevent water loss), which indirectly affects uptake efficiency after refilling of the soil profile. Water availability also affects transpiration rates (Taiz and Zeiger, 2010). Water deficit can thus affect the carbohydrate status of the tree by decreasing current photosynthetic production (Naschitz et al., 2010). Since root growth and maintenance are highly dependent on current photosynthesis (Pn) (Horwath et al., 1994; Schupp and Ferree, 1990), root growth will be affected indirectly as well.

Low soil water availability or water stress (-200 kPa) can reduce final biomass and increase specific root length (SRL), as well as the carbon cost of root maintenance in apple trees (‘Mutsu’/M9)(Psarras and Mervin, 2000). The root:shoot ratio of apple trees generally increases due to water stress, although the reduction in shoot growth varies according to rootstock (Psarras and Mervin, 2000). The reduction in shoot growth for more vigorous (M.111) rootstocks was also relatively greater than for dwarfing (M9) rootstocks in field grown ‘Empire’ apples (Psarras and Mervin, 2000). Yao et al. (2009) reported higher root mortality rates, in addition to reduced rates of root emergence, when hot and dry conditions prevailed. Rhizosphere respiration was also reduced in two different rootstocks (M.9 and M.111) under water stress (-80 kPa and -200 kPa) conditions (Psarras and Mervin, 2000). The reduced root respiration was attributed to a lower respiratory demand by the roots and/or the reduced

photosynthate supply from the leaves resulting from water stress conditions (Naschitz et al., 2010; Psarras and Mervin, 2000).

Fine root distribution also differs considerably between well-watered and water-stressed trees (Green and Clothier, 1999), where finer and/or roots with lower tissue density occur as a result of high (-200 kPa) water stress (Psarras and Mervin, 2000). In addition, transpiration rates and sap flow of deeper rooted, well-watered trees had a slight response to irrigation, whereas stressed trees with fine roots concentrated in the upper 5 cm soil, increased sap flow rates up to five following irrigation (Green and Clothier, 1999).

It is not only a lack of water which influences roots, but soil water levels above field capacity ultimately undermine oxygen replenishment within the soil (Gregory, 2008) and compromise root respiration by inhibiting air flux, which becomes a limiting factor for root growth (Gregory, 2008). In addition, various other factors are strongly influenced by soil water. The degree of fluctuations in root zone temperature is influenced by soil water dynamics and roots are much more sensitive to sudden fluctuations in temperatures than shoots (Fageria, 2013; Nielsen, 1974; Pregitzer et al., 2000). Soil water content influences the thermal properties of the soil with higher water content lowering the degree of temperature fluctuation (Faget et al., 2013). Water is also the vector for nutrient suspension, flow and uptake. Therefore, optimizing soil water dynamics reduces stress from many other factors, besides the direct effects of water stress (Fageria, 2013; Gregory, 2008).

Soil aeration

The concentration of O_2 and CO_2 in soil pores are inversely related (Marschner, 1995). Root respiration depletes O_2 in soil pores, while producing CO_2, with a resulting increase in the CO_2:O_2 ratio of the gas in the soil pores. Root development is highly dependent on O_2 availability due to the high respiration rates of roots (Marschner, 1995; Psarras et al., 2000). If the removal rate of CO_2 and the supply of O_2 in the soil matrix are inadequate, root growth becomes restricted due to inadequate respiration which also reduces nutrient uptake (Gregory, 2008; Psarras et al., 2000). Not only is inadequate O_2 deleterious to root growth, but also the presence of high CO_2 concentration and its associated toxic products can directly inhibit root growth (Fageria, 2013; Gregory, 2008). Root elongation rate furthermore decreases when subjected to waterlogged conditions due to the disruption in gas flow. A reduced root

elongation rate also has been correlated to decreasing O_2 concentration and diffusion rate during waterlogged conditions (Blackwell and Wells, 1983; Gregory, 2008).

Rooting depth also becomes restricted by the increasing CO_2:O_2 ratio that occurs with soil depth (Marschner, 1995), which is due to an increased soil density (restricting air movement), longer periods of water saturation and the greater distance for gases to diffuse between the soil and the atmosphere (Marschner, 1995). A high ethylene concentration further exacerbates the growth inhibiting effects of low O_2 under water-saturated conditions (Marschner, 1995). Ethylene production by the roots is usually increased under low O_2 concentration (Taiz and Zeiger, 2010). As the radial diffusion of ethylene away from the root is impaired by the water around the roots, it accumulates more during waterlogged conditions, than under aerated soil conditions (Marschner, 1995). In addition, ethylene production by microorganisms further adds to ethylene accumulation in a poorly aerated rhizosphere (Marschner, 1995).

Root morphology, development and longevity

The primary functions of the root system are water absorption, nutrient acquisition, anchorage, storage of metabolites and the synthesis of growth regulators or phytohormones (Ma et al., 2013; Osmont et al., 2007). The efficiency of functions, such as water and nutrient uptake, are influenced by specific morphological features, such as casparian band development, root browning and SRL (Baldi et al., 2010; Eissenstat et al., 2000; White, 2001). The casparian band consists primarily of lignin and to a lesser extent suberin (White, 2001), the deposition of which is also influenced by environmental conditions. Suberin is a lipid polymer with wax-like hydrophobic properties and is deposited in endodermal cells to form a barrier to water and solute flow (Taiz and Zeiger, 2010; White, 2001). Endodermal cells develop casparian bands as they mature, which restrict and may, over time, block apoplastic solute movement into the stele, as suberification and lignification advances. It is necessary for older regions of the root to be impermeable to apoplastic solute movement to some extent to facilitate bulk flow from more distal parts of the roots to the trunk (Taiz and Zeiger, 2010). The fully developed endodermal layer therefore allows the root to regulate its internal conditions by channelling solute movement through the symplasm of the endodermis, thereby also preventing backflow of ions into the apoplast of the cortex or back into the soil environment (Enstone et al., 2003; Taiz and Zeiger, 2010). The greatest potential for apoplastic flow occurs in actively growing root tips where the casparian bands are absent, as well as in young parts of the roots where the

casparian bands are under-developed (Marschner, 1995; White, 1998, 2001). White roots primarily function as absorbing roots as they partially consist of juvenile cortical tissue (Nightingale, 1935). The juvenile cortex is associated with a high activity in nutrient and water acquisition (White, 2001). In addition to nutrient absorption, these actively absorbing white roots are also associated primarily with cytokinin synthesis (Ma et al., 2013). Depending on environmental conditions (especially soil temperature), newly developed white roots can remain white for a time span ranging from days to weeks, before browning in transition to a potential second order root (Nightingale, 1935). Generally, higher soil temperatures, dry soil conditions and high pathogen levels in the soil increase the rate of root browning (Eissenstat et al., 2000; Wells and Eissenstat, 2003).

The endodermis itself develops in three stages, where endodermal cell wall thickening is followed by suberification and further lignification as the root develops (Enstone et al., 2003; White, 2001). This in turn leads to decreased permeability with each successive developmental stage (Gregory, 2008; White, 2001). In an apple tree root, the formation of the casparian band occurs about 1 cm from the root tip at soil temperatures of 24 °C, whereas at lower temperatures (12 °C), casparian band formation was only noticed 5 cm from the root apex while the cortex remained white. At temperatures above 24 °C, the maturity of the endodermis was much more advanced to the point where the cortex was brown and often dead (Nightingale, 1935). Visually, root maturation in apple and other species is associated with colour changes from white to brown (Baldi et al., 2010; Ma et al., 2013; Wells and Eissenstat, 2001). Morphologically and physiologically, root maturation is the process of shedding the cortex, while the endodermal cells become suberized and lignified and root metabolic activity decreases (Baldi et al., 2010; Comas et al., 2000; Eissenstat et al., 2000; Marschner, 1995; White, 2001). However, root browning is not always directly linked to suberization and may be caused by senesence (Wells and Eissenstat, 2003). If the browning is due to pigmentation, caused by phenolic accumulation, the root is undergoing maturation rather than senescence (Wells and Eissenstat, 2001). The white to brown colour change associated with maturing roots is accompanied by a decrease in diameter of the root as cortical tissue is sloughed off (Psarras et al., 2000). This has implications for root size distribution estimates, as the fine root fraction tends to increase as roots go through their first maturation phase.

After root maturation, most fine roots of woody plants remain brown and do not become woody through secondary growth (Eissenstat and Achor, 1999) and are therefore unable to increase in

diameter with age, as they are short lived (Eissenstat et al., 2000). Root functionality shifts with the onset of secondary radial growth, which usually occurs after harvest in apple (Terblanche, 1986). According to Eissenstat et al. (2000), larger older roots have a multifunctional nature, whereas unbranched, small diameter roots specialize in acquiring water and nutrients. Larger branched roots and lignified fine roots perform functions such as anchorage, storage of reserves, solute transport as well as lateral root production (Eissenstat et al., 2000; Ma et al., 2013). Differences in the degree of maturity and secondary growth, as well as the arrangement of cell types, are responsible for the functional differences between roots of the same plant (Gregory, 2008). Usually, roots that undergo secondary radial growth have increased potential for long-term survival. Also, a significantly higher longevity has been found for roots with dependent laterals compared to unbranched roots (Eissenstat et al., 2000). Furthermore, tree age or maturity affects the percentage of white roots that become permanent structural roots, as younger apple trees retain more of their newly formed roots compared to older bearing trees (Hughes and Gandar, 1993).

Root turnover

In general, fine roots of deciduous trees are shorter lived than for evergreen trees and therefore have a higher turnover rate (Psarras et al., 2000; Wells and Eissenstat, 2001). The general life span for apple tree roots can range from days to years (Ma et al., 2013). Root turnover has implications for the apple tree carbon balance, as up to 85 % of annually produced root tissue may be lost due to death and cortex breakdown (Palmer, 1988). Apple root growth consumes a major portion of the available carbon for metabolic activity and high turnover rates (Psarras et al., 2000). Maximum rates of root turnover have been found to occur towards the end of the growing season or late summer (Psarras et al., 2000). In deciduous forests, root turnover may even exceed the cost of leaf turnover in terms of biomass (Kramer and Boyer, 1995).

In mature, deciduous hardwood forests, finer (<0.05 mm), as well as shallower roots, have a higher turnover rate (Joslin et al., 2006). Yao et al. (2009) also reported that the success of apple root survival during winter was positively correlated to root diameter, with larger roots having a better survival rate. However, in another study involving apple root longevity, no consistent correlation between longevity and root diameter was found (Psarras et al., 2000). These authors found that roots thicker than 1 mm had a higher tendency to disappear during the growing season, whereas finer roots (<1 mm) overwintered more often. In addition to these

contrasting findings, remarkable differences in terms of lifespan, overwintering ability and maturation within the fine root class (<1mm diameter) have also been reported for apples (Wells and Eissenstat, 2001). It seems therefore that root diameter is a poor predictor of root lifespan. Rather, fine roots bearing daughter roots and having lower maintenance respiration, lower nitrogen (N) concentration, mycorrhizal colonization and a low SRL (higher tissue density) generally have longer lifespans (Eissenstat et al., 2000). Short-lived roots therefore show higher absorption ability due to high respiration and SRL (Eissenstat et al., 2000).

Root mortality seems to be quite complex and unpredictable in apple trees due to the interaction of environmental, cultural and endogenous factors (Yao et al., 2009). Apple roots are considered highly adaptive to different environmental conditions (Eissenstat et al., 2000), although they are also easily shed when they become inefficient. This can occur when the soil becomes too dry or soil temperatures are too high and may also be shed if the root is located in an infertile patch of soil (Eissenstat et al., 2001). Root growth behaviour may also respond differently to crop load, depending on environmental conditions (Yao et al., 2009). Roots respond to the interactions of these factors by altering various morphological aspects, such as root emergence, mortality, median life span, as well as cumulative root numbers, in different ways. A lower root mortality and higher root median life span was observed during a heavy crop year, than during a light crop year in apple (Yao et al., 2009), even though high fruit yields tend to inhibit dry matter partitioning to the root system (Palmer, 1992). Root growth during the lighter crop year did, however, have higher cumulative root numbers than the heavy crop year (Yao et al., 2009), indicating higher assimilate availability during the light crop year. The lower root mortality rates in the high production year compared to the low production year seem to result from both external and internal conditions. The climatic conditions of the high production year were cooler and wetter (1 day with maximum temperatures > 32° C and frequent evenly distributed rainfall) which seemed to promote root longevity (Yao et al., 2009). These conditions also promoted root emergence and growth in this study (Yao et al., 2009), as root mortality rates are usually increased by higher temperatures and low soil water levels, as previously stated (Eissenstat et al., 2000).

According to Psarras et al. (2000), root turnover increases as root emergence declines. After new roots emerge, the cycle of senescence and decay, with eventual disappearance, starts (Psarras et al., 2000). At the onset of the growing season, however, root mortality is generally minimal (Psarras et al., 2000; Yao et al., 2009). Newly formed white roots have two

possibilities in terms of long-term development. After emergence they can remain white for some time and may even develop lateral roots themselves and eventually senesce and decay, completing a short life cycle. Alternatively, the newly formed white roots become brown and mature. These mature roots are then able to over-winter and continue growth in the following season by producing lateral roots. However, even mature roots may naturally decay and disappear, also having a short life cycle. Premature disappearance or decay of roots in any developmental phase is therefore possible. Extremely high temperatures, pests, diseases, drought stress and waterlogged soil conditions can all cause roots to die prematurely (Eissenstat et al., 2000; Psarras et al., 2000).

Differences in root longevity also exist between different rootstocks, but these differences only become noteworthy with the onset of cropping (Yao et al., 2006). This corresponds to other findings for young non-bearing trees, which suggests that continuous root growth with a lower mortality rate is needed for root system establishment (Atkinson and Wilson, 1980; Hughes and Gandar, 1993).

Specific root length

Total root length (TRL) of a root system is related to its ability to acquire water and nutrients. Root length is a genotypically controlled trait that is significantly influenced by environmental conditions, such as water, temperature and fertility, especially fertilisers containing N and P (Fageria, 2013). The root length to mass ratio, or SRL, is an important indicator for studying fine root morphology, C usage and longevity (Eissenstat and Achor, 1999; Fageria, 2013) and is defined for individual roots as root length (cm) per root dry mass (g). Certain physiological, morphological and anatomical variations between different roots can be correlated to differences in SRL (Eissenstat et al., 2000). A high SRL is also characteristic of apple roots (Eissenstat et al. 2000). However, information regarding the SRL in apple specifically is limited (Psarras and Mervin, 2000). Nevertheless, plants with a high SRL appear to absorb water and nutrients better than plants with a low SRL (Fageria, 2013), as SRL is positively correlated to root hydraulic conductivity and rates of root proliferation (Eissenstat et al., 2000). Low tissue density means less secondary wall thickening of the exodermis through lignification, hence the benefits for solute uptake of root with a high SRL (Eissenstat et al., 2000). Tree-soil water relations can therefore be affected by SRL (Psarras and Mervin, 2000). Higher SRL and a greater number of fine roots, were reported for apple under high water stress

conditions (Psarras and Mervin, 2000), indicating a morphological adaptation mechanism to water stress. Apple roots seem to have a relatively high degree of adaptability as apple roots show greater differences in SRL, tissue density and diameter when grown in different environments, compared to citrus (Eissenstat et al., 2000). However, according to Eissenstat (1991), a high SRL is also associated with lower tissue density and a smaller average root diameter which corresponds to a shorter root lifespan, as compared to citrus (Eissenstat and Achor, 1999; Eissenstat et al., 2000), but is also a characteristic of higher plasticity and adaptability (Eissenstat et al., 2000). Citrus rootstocks with a high SRL were also reported to absorb water and proliferate more rapidly than low SRL rootstocks (Eissenstat, 1991). The lower carbon cost of building roots with a higher SRL helps to explain the shorter lifespan but higher efficiency of producing roots with a high SRL under water stress conditions (Eissenstat, 1991). The low tissue density of roots with a high SRL also tends to make roots more succulent and fragile (Eissenstat et al., 2000).

Root maturation and nutrient uptake efficiency

The morphological changes associated with root development have implications for nutrient uptake efficiency. Actively growing white roots are mostly equipped for nutrient absorption, organic compound and cytokinin biosynthesis (Baldi et al., 2010; Ma et al., 2013; Marschner, 1995; White, 2001). Water uptake is also higher in root apical regions i.e. regions where the path to the stele is unobstructed by casparian bands (Marschner, 1995; Taiz and Zeiger, 2010). However, rapid solute uptake and translocation rates often occur at more distal regions from the apex, where xylem is more mature (Wells and Eissenstat, 2003). Roots at all stages of development are therefore able to actively absorb nutrients and water, including woody basal zones (Marschner, 1995; Zimmerman et al., 1971). Small tears in the endodermal layer, due to the expansion of the stele or the emergence of lateral roots above the maturation zone, where cortical cells start to senesce, create apoplastic pathways that may explain the unexpected higher solute uptake in the lignified part of the root (Nightingale, 1935; Zimmerman et al., 1971). In addition, “passage cells” may also help explain the potential for higher uptake in lignified regions of the root, as they are thin walled and unsuberized cells, directly exposed to the environment after cortical senescence has taken place (Enstone et al, 2003; White, 2001).

According to Baldi (2010), the white to brown colour change in roots is associated with the deposition of suberin; with white roots in peach having a higher N uptake efficiency, in addition

to higher respiration rates compared to brown root tissue. Suberization and lignification of the endodermis only comes with maturity, changing the root's morphology above the elongation zone (Marschner, 1995). As discussed previously, below the maturation zone, the apoplastic pathway to the xylem is unrestricted for solute movement as the cell walls of the endodermal cell layer have not been lignified and completely suberized (Taiz and Zeiger, 2010; White, 2001). Furthermore, respiration rates are much higher in the meristematic regions where the cells have significantly more plasmodesmata and are non-vacuolated (Taiz and Zeiger, 2010). Symplastic active nutrient uptake is therefore also much higher in these regions, in addition to the continuous apoplastic pathways available in the root tip (Marschner, 1995). The higher uptake rates in the apical zone may also be ascribed to the higher nutrient availability of uncolonized soil into which the root grows (Taiz and Zeiger, 2010).

The unsuberized part of the root tip varies in length between species ranging between a few millimetres to several centimetres (Taiz and Zeiger, 2010). According to the anatomical study by Nightingale (1935), signs of casparian band formation become visible approximately 1 cm from the root tip in apple ("Stayman Variety") (Nightingale, 1935). However, the successive stages of casparian band formation described by White (2001), and anatomical observation of incomplete formation of casparian bands 1 cm from the root tip, suggests that an uptake rate gradient occurs along the root axis. The various rates of ion uptake along the root axes also depends on the particular nutrient absorbed (Marschner, 1995), with a tendency for nutrient uptake rates in general to decline further away from the root apex (Marschner, 1995; White, 2001). Heterogeneity therefore exists along the root axis of a developing root in terms of nutrient uptake and tissue composition, mainly due to differences in morphology, biochemistry and tissue age (Marschner, 1995; Wells and Eissenstat, 2003; White, 2001). In particular, the uptake of Ca is much higher in the apical root zone, where tissues are unsuberized, than in the basal regions of the root where tissues are more mature (Marschner, 1995; White, 2001). Marschner (1995) reports significantly higher uptake of Ca up to 3 cm from the tip of maize roots and still higher rates at 6 cm, compared to 12 cm. The Ca requirement of the root apical meristem is dependent on direct uptake, mainly due to the phloem immobility of Ca and also because of the under-developed sieve elements in this developing region of the root (Gregory, 2008; Marschner, 1995). The Ca uptake in basal root zones can be particularly low due to the exchange mechanism of Ca uptake, which is also further restricted by the fully developed casparian bands and tertiary cell walls (Marschner, 1995).

Root growth and whole-tree physiology

Root – Shoot – Fruit interaction

The root system is directly connected to the atmosphere through the shoots, and is dependent on the carbohydrates resulting from Pn (Taiz and Zeiger, 2010). Thus, roots are affected by atmospheric conditions, including radiation intensity (Marschner, 1995). Roots are therefore not only directly affected by the climate through soil temperature and water responses, but also indirectly through the effects on shoot processes such as Pn and sink interactions (Farrar and Jones, 2000; Kozlowski, 1992; Palmer, 1992).

Initially, root and shoot systems were believed to be inter-related through a balance in size or mass. However, more recent experiments revealed that a unique equilibrium develops between roots and shoots in terms of efficiency depending on the environmental conditions (Farrar and Jones, 2000; Gregory, 2008). The concept of "functional equilibrium" demonstrates that root and shoot growth is balanced, so that the growth of the one to which an essential element is limited, becomes favoured (Gregory, 2008). Water stressed apple trees, for example, have a significantly higher root-shoot ratio (Psarras and Mervin, 2000), essentially optimizing the chances for water uptake (Green and Clothier, 1999), while restricting transpiration losses by reducing above ground growth. Furthermore, growth maintains the favoured functional ratio when the tree's root:shoot ratio is disturbed by either root, or shoot removal (Fumey et al., 2011; Gregory, 2008) with Schupp and Ferree (1990) reporting an increase in apple rootstock growth in response to root pruning. The root:shoot ratio is also influenced by environmental conditions, such as fertility, where significantly higher root:shoot ratios occur under low compared to high fertility conditions in apple (Rogers and Head, 1969). Similarly, when potted plants are moved to a different environment (eg. higher or lower light conditions) they generate a new unique root-shoot ratio (Gregory, 2008). Therefore, the root and shoot system of a tree respond to each other based on the efficiency of their performance in obtaining their specific suite of resources. Lower root:shoot ratios are adequate for plants receiving optimal levels of nutrients, water and oxygen (Fageria, 2013; Gregory, 2008; Taiz and Zeiger, 2010).

Tree carbon balance

Assimilate partitioning and sink competition play a major role in active root growth dynamics (Maggs, 1963; Schupp and Ferree, 1990; Yao et al., 2006). The photosynthetic source-sink interaction is considered much more complex in deciduous tree species than in annual plants (Cheng et al., 2008). In mature apple trees, the source organ comprises the leaves, while the total sink activity of the tree is divided into fruits, shoots and leaves, roots, secondary growth and reserves (Cheng et al., 2008; Palmer, 1992). Fruit development in apple trees represent a large portion of the fixed photosynthates. Up to 70 % of annual dry matter production in bearing apple trees can be in the form of fruit (Duan et al., 2008; Heim et al., 1979; Palmer, 1988). Assimilate partitioning to apple roots may therefore vary between 5 – 41 %, depending on fruit sink strength (Heim et al., 1979). Interestingly, root growth becomes more periodic as newly established trees come into bearing (Yao et al., 2006). Young, newly established trees show potential for continuous root and shoot growth throughout the season if trees are not water stressed (Atkinson and Wilson, 1980; Cripps, 1970). Water stress on young trees during summer resulted in the cessation of root growth until autumn rainfall stimulated the return of rapid root growth (Cripps, 1970). This persistent potential observed in young trees for continuous root growth is related to endogenous factors. In young trees, due to the absence of reproductive structures, a larger portion of the annually obtained assimilates are available for root growth (Flore and Layne, 1999). Young trees also have an inherent tendency to establish a root system for anchorage by initially growing at a lower RLD in the first four years (Hughes and Gandar, 1993). In addition, the root system of young trees are much closer to the source of photosynthates and represent a larger portion of the total sink demand than in older bearing trees (Flore and Layne, 1999; Heim et al., 1979). Furthermore, root growth dynamics tend to alternate with active shoot growth phases (Ma et al., 2013). Periodic phases in root and shoot growth have been shown to occur asynchronously in several species (Fumey et al., 2011). However, Cripps (1970) reported that root and shoot growth occurred concurrently for both bearing and non-bearing apple trees.

Cropping reduces root growth

Active root growth in apple is affected by crop load and can be linked to long-term trends in tree performance. Yao et al. (2009) observed more root growth in a light crop year compared to the following year, which had a heavy crop load possibly indicating sink competition (Yao et al., 2009). Similarly, for younger apple trees, fine root growth was strongly reduced in the first bearing year compared to the previous non-bearing year, irrespective of pre-plant

treatment or rootstock type (Yao et al., 2006). They ascribe the reduced root growth to increased competition for carbohydrates by shoots and especially fruit. Palmer (1992) also found that carbon partitioning to roots varied according to crop load, with higher crop loads resulting in less photosynthates being allocated for root development (Maggs, 1963; Palmer, 1992). A change in phenology also occurs with the shift from non-bearing to bearing growth in fruit trees (Schupp and Ferree, 1990), as reproductive processes make assimilate partitioning more complex. Fruit development therefore becomes the dominant sink, consequently making root growth distinctly periodic (Flore and Layne, 1999; Yao et al., 2006).

Photosynthesis and root growth

Sink demand is a key regulatory component in photosynthetic adjustment (Paul and Foyer, 2001). The level of photosynthetic machinery (chloroplast protein and pigment composition), which determines photosynthetic capacity, is flexible and changes according to the resource economy of the plant (Paul and Foyer, 2001). Sink demand for reduced high-energy molecules, by the various developing tissues, depends on their metabolic activity and storage needs (Marschner, 1995). This source-sink based signal transduction network overrides the direct CO_2/light based regulation of photosynthetic capacity (Paul and Foyer, 2001). The consequence of either adding or removing a sink organ, results in the up- or down-regulation of photosynthetic capacity (Cheng et al., 2008; Fan et al., 2010; Palmer, 1992). For example, it was shown that leaf photosynthetic rate increases with higher cropping intensity (Palmer, 1992), or decreases in response to an interruption of the root sink through girdling (Cheng et al., 2008).

A substantial proportion of annually produced photosynthates are allocated for fine root development (Eissenstat et al., 2000). Therefore, the temporary removal of the root system sink in apple via girdling, results in a decrease in Pn (Cheng et al., 2008; Fan et al., 2010). The removal of the root sink in apple, consequently leads to carbohydrate accumulation at the source (Fan et al., 2010). Stomatal conductance (G_s) and transpiration rate (E) also decrease in conjunction with Pn, as a result of the low carbohydrate demand from the roots (Fan et al., 2010). The exact pathways by which photosynthetic adjustments are regulated, particularly in response to low sink demand is, however, not clear (Fan et al., 2010). Nevertheless, Pn, G_s and E responses to girdling appear to be temperature dependent. Trees grown under higher temperatures were more responsive in terms of Pn, G_s and E reductions after girdling (Fan et

al., 2010). Pn, in contrast, was not regulated by a direct end-product feedback mechanism (Cheng et al., 2008; Fan et al., 2010), but was more likely due to stomatal limitation (via decreased leaf transpiration) and non-stomatal limitation (via increased leaf temperature) for trees grown under lower and higher temperatures (Fan et al., 2010). Besides differences in apple variety, leaf age and sink demand, leaf photosynthesis also differ between rootstocks as trees on vigorous rootstocks tend to have higher photosynthetic rates (Schechter et al., 1991).

Conclusion

The understanding and quantification of root growth periodicity in apple is of importance for scheduling fertilizer applications. Active root growth is marked by the production of white roots which develop laterally from more mature brown roots. Compared to brown roots, the properties of white roots are much more suited for optimal nutrient uptake rates (Baldi et al., 2010; Eissenstat et al., 2001; Ma et al., 2013; Marschner, 1995; Wells and Eissenstat, 2003; White, 2001). The timing of fine root growth in woody perennials, however, does not follow a particular phenological pattern and is therefore often difficult to predict (Atkinson and Wilson, 1980; Rogers and Head, 1969). Understanding the factors controlling root growth is therefore important. It is evident that the periodic nature of root growth in fruit trees is a function of both endogenous tree processes interacting with the environment, as well as the direct effect of environmental conditions on root growth (Flore and Layne, 1999; Rogers and Head, 1969; Yao et al., 2006). However, roots are able to grow at a relatively wide range of soil temperatures, although temperature has a qualitative effect on short term root characteristics and affects root mass in the long term (Kasper and Bland, 1992; Nightingale, 1935; McMichael and Burke, 1998). Similarly, changes in soil water content affect root characteristics and growth rate, rather than the dynamics of root activity and only under extreme conditions does root growth cease entirely (Bevington and Castle, 1985). Root growth cycles are therefore more likely determined by the dynamics of carbon partitioning and the relationship between sinks (Côté et al., 1998; Maggs, 1963; Rogers and Head, 1969). Pn is the primary source providing photosynthates to the root system for growth, nutrient assimilation and maintenance respiration (Marschner, 1995; Taiz and Zeiger, 2010). Due to the limited time available for aerial deciduous tree parts to complete their developmental cycle, they are favoured in terms of resource allocation during peak demand (Flore and Layne, 1999). On the other hand, a root growth cycle is marked by a production of white roots which are typically very short lived and generally serve a short term purpose (Withington, 2005). The dominant role of endogenous resource dynamics over

environmental parameters in controlling root growth is also evident in the different root growth potentials for bearing and non-bearing trees. Bearing fruit trees typically show periodic root growth patterns (Yao et al., 2006), whereas young trees show potential for continuous growth, given suitable environmental conditions (Cripps, 1970). The periods of viable environmental conditions suitable for root growth, however, vary according to climatic regions and cultural practices (Atkinson and Wilson, 1980; Cripps, 1970). In certain regions therefore, saturated soil water conditions or extreme soil temperatures may be the prime parameters affecting the timing of a root growth cycle (Psarras et al., 2000; Kuhns et al., 1985). In other climatic regions, environmental conditions only seldom become a limiting factor to root growth. Root growth dynamics of fruit trees, however, will always be strongly influenced by whole-tree carbon balance (Atkinson and Wilson, 1980; Flore and Layne, 1999).

References

Atkinson, D. and Wilson, S.A. 1980. The growth and distribution of fruit tree roots: some consequences for nutrient uptake., (eds.) Atkinson, D., Jackson, J. E., Sharples, R. O. and Waller, W. M. *Mineral nutrition of fruit trees,* Butterworths publishers, 1980, 137-150.

Baldi, E., Wells, C. E. and Marangoni, B. 2010. Nitrogen absorption and respiration in white and brown peach roots. Journal of Plant Nutrition 33, 461-469.

Ben-Asher, J., Cardon, G., Peters, D., Rolston, D.E., Biggar, J.W., Phene, C.J. and Ephrath, J.E. 1994a,b. Determining root activity distribution by measuring carbon dioxide fluxes. Soil Science Society of America Journal 58, 926– 934.

Bergh, O. 1990. Effect of time of hand-thinning on apple fruit size. South African Journal of Plant and Soil 7(1), 1-10.

Bevington, K. B. and Castle, W. S. 1985. Annual root growth pattern of young citrus trees in relation to shoot growth, soil temperature, and soil water content. Journal of the American Society for Horticultural Science 110(6), 840-845.

Blackwell, P.S. and Wells, E.A. 1983. Limiting oxygen flux densities for oat root extension. Plant and Soil 73, 129-139.

Burke, M. K. and Raynal, D. J. 1994. Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. Plant and Soil 162(1), 135-146.

Cheng, Y., Arakawa, O., Kasai, M. and Sawada, S. 2008. Analysis of reduced photosynthesis in the apple leaf under sink limited conditions due to girdling. Japan. Society of Horticultural Science 77(2), 115-121.

Comas, L. H. Bouma, T. J. and Eissenstat, D.M. 2002. Linking root traits to potential growth rate in six temperate tree species. Oecologia 132, 34 – 43.

Comas, L. H., Eissenstat, D. M., and Lakso, A. N. 2000. Assessing root death and root system dynamics in a study of grape canopy pruning. *New Phytologist 147*(1), 171-178.

Côté, B., Hendershot, W. H., Fyles, J. W., Roy, A. G., Bradley, R., Biron, P. M. and Courchesne, F. 1998. The phenology of fine root growth in a maple-dominated ecosystem: relationships with some soil properties. Plant and Soil 201(1), 59-69.

Cripps, J. E. L. 1970. A seasonal pattern of apple root growth in Australia. Journal of Horticultural Science 45, 153-161.

Duan, W., Fan, P. G., Wang, L. J., Li, W. D., Yan, S. T. and Li, S. H. 2008. Photosynthetic response to low sink demand after fruit removal in relation to photo inhibition and photo protection in peach trees. Tree Physiology 28, 123-132.

Eissenstat, D. M. and Achor, D. S. 1999. Anatomical characteristics of roots of citrus rootstocks that vary in specific root length. New Phytologist 141(2), 309-321.

Eissenstat, D. M., Lakso, A. N. Neilsen, D., Neilsen, G. H. and Smart, D. R. 2006. Seasonal patterns of root growth in relation to shoot phenology in Grape and Apple. Acta Horticulturae 721, 21-26.

Eissenstat, D. M., Wells, C. E. Yanai, R. D. and Whitbeck, J. L. 2000. Building roots in a changing environment: implications for root longevity. Review, New Phytologist 147, 33-42.

Eissenstat, D. M., Wells, C. E. and Wang, L. 2000. Root efficiency and mineral nutrition in apple. In IV International Symposium on Mineral Nutrition of Deciduous Fruit Crops 564 (pp. 165-183).

Eissenstat, D.M. 1991. On the relationship between specific root length and the rate of root proliferation: a field study using Citrus rootstocks. New Phytologist. 118, 63-68.

Enstone, D. E., Peterson, C. A. and Ma, F. 2003. Root endodermis and exodermis: structure, function, and responses to the environment. Journal of Plant Growth Regulation 21,

335–351.

Fageria, N. K. 2013. The role of plant roots in crop production. Taylor and Francis group, LLC, 8-14, 20-33, 159,185-191, 323.

Faget, M., Blossfeld, S., Jahnke, S., Huber, G., Schurr, U. and Nagel, K. A. 2013. Temperature effects on root growth. Plant roots: The hidden half, (eds). Eshel, A. and Beeckman, T, 4th edition, Taylor and Francis Group, CRC press, 2013, 31(1-8).

Fallahi, E. 1994. Root physiology, development and mineral uptake. p.19-30. In: A.B. Peterson and R.G. Stevens (eds.), Tree Fruit Nutrition: A Comprehensive Manual of Deciduous Tree Fruit Needs. Good Fruit Grower, Yakima, Washington, USA.

Fan, P. G., Li, L. S., Duan, W., Li, W. D. and Li, S. H. 2010. Photosynthesis of young apple trees in response to low sink demand under different air temperatures. Tree Physiology 30, 313-325.

Farrar, J. F. and Jones, D. L. 2000. The control of carbon acquisition by roots. New Phytologist 147(1), 43-53.

Flore, J. A. and Layne, D.R. 1999. Photosymilate production and distribution in Cherry. Horticultural Science 34(6), 1015 -1019.

Fukuzawa, K., Dannoura, M. and Shibata, H. 2012. Fine root dynamics and root respiration. Measuring roots, Mancuso, S (ed.), Springer- Verlag Berlin Heidelberg 2012, Chapter 15, 341-356.

Fumey, D., Lauri, P., Guedon, Y., Godin, C. and Costes, E. 2011. How young trees cope with removal of whole parts of shoots: An analysis of local and distant responses to pruning in 1-year-old apple (*Malus*× *Domestica*; Rosaceae) trees. American Journal of Botany 98 (11), 1737-1751.

Gluszek, S., Paszt, L. S., Sumorok, B., Derkowska, E. and Kozera, R. 2013. Application of the minirhizotron technique to studying the roots of fruit plants. Advances in Science and

Technology Research Journal 7(18), 45–53.

Green, S. R. and Clothier, B. E. 1999. The root zone dynamics of water uptake by a mature apple tree. Plant and Soil 206, 61-77.

Greer, D.H., Wünsche, J.N., Norling, C.L. and Wiggins, H.N. 2005. Root-zone temperatures affect phenology of bud break, flower cluster development, shoot extension growth and gas exchange of 'Braeburn' (*Malus domestica)* apple trees. Tree Physiology 26, 105–111.

Gregory, P. J. 2008. Plant roots: Growth, activity and interaction with soils. Blackwell Publishing Ltd. p. 5-7, 64-65, 131-135, 149-152.

Head, G.C. 1967. Effects of seasonal changes in shoot growth on the amount of unsuberized root on apple and plum trees. J. Hort. Sci. 42:169-180.

Heim, G., Landsberg, J.J., Watson, R.L. and Brian, P. 1979. Eco-Physiology of apple trees: Dry matter production and partitioning by young Golden delicious trees in France and England. Journal of Applied Ecology 16, 179-194.

Hodge, A., Berta, G., Doussan, C., Merchan, F. and Crespi, M. 2009. Plant root growth, architecture and function. Plant and Soil 321, 153–187.

Horwath, W. R., Pregitzer, K. S. and Paul, E. A. 1994.^{14}C Allocation in tree-soil systems. Tree Physiology 14, 1163-1176.

Hughes, K. A. and Gandar, P. W. 1993. Length densities, occupancies and weights of apple root systems. Plant and Soil 148, 211-221.

Joslin, J. D., Gaudinski, J. B., Torn M. S., Riley W. J. and Hanson, P. J. 2006. Fine root turnover patterns and their relationship to root diameter and soil depth in a ^{14}C-labeled hardwood forest. New Phytologist 172, 523 –535.

Joslin, J. D., Wolfe, M. H. and Hanson, P. J. 2001. Factors controlling the timing of root

elongation intensity in a mature upland oak stand. Plant and Soil 228(2), 201-212.

Kaspar, T. C. and Bland, W. L. 1992. Soil temperature and root growth. Soil Science 154(4), 290-299.

Kozlowski, T. T. 1992. Carbohydrate sources and sinks in woody plants. The Botanical Review 58(2), 107-222.

Kozlowski, T. T., Kramer, P. J. and Pallardy, S. G. 1991. The physiological ecology of woody plants. Academic press, inc. 180-182.

Kramer, P. J. and Boyer, J. S. 1995. Water relations of plants and soils. Academic press, Inc.

Kuhns, M. R., Garrett, H. E., Teskey, R. O. and Hinckley, T. M. 1985. Root growth of black walnut trees related to soil temperature, soil water potential, and leaf water potential. Forest Science 31(3), 617-629.

Li, K.T., Lakso, A.N., Piccioni, R. and Robinson, T., 2003. Summer pruning effects on fruit size, fruit quality, return bloom and fine root survival in apple trees. Journal of Horticultural Science & Biotechnology 78(6), 755-761.

Ma, L., Hou, C. W., Zhang, X. Z. Li, H. L., Han, De G., Wang, Y. and Han, Z. H. 2013. Seasonal growth and spatial distribution of Apple tree roots on different rootstocks or interstems. Journal of American Society of Horticultural Science 138(2), 79–87.

Maggs, D. H. 1963. The reduction in growth of apple trees brought about by fruiting. Journal of Horticultural Science 38(2), 119-128.

Marchner, H. 1995. Mineral nutrition of higher plants second edition. London. Academic press, 7-12, 63-70, 484-500, 508-513.

McManus, M. T. and Veit, B. E. 2002. Meristematic tissues in plant growth and development. Edited by M.T. McManus and B.E. Veit. 2002. Sheffield academic press. Chapter 9, p. 279-291.

McMicael, B. L. and Burke, J. J. 1998. Soil temperature and root growth. Hortscience, Vol. 33(6), 947-951.

Miqueloto, A., do Amarante, C. V. T., Steffens, C. A., dos Santos, A. and Mitcham, E. 2014. Relationship between xylem functionality, calcium content and the incidence of bitter pit in apple fruit. Scientia Horticulturae 165, 319-323.

Montagnoli, A., Di Iorio, A., Terzaghi, M., Trupiano, D., Scippa, G. S. and Chiatante, D. 2014. Influence of soil temperature and water content on fine-root seasonal growth of European beech natural forest in Southern Alps, Italy. European Journal of Forest Research 133(5), 957-968.

Nambiar, E. K. S. 1983. Root development and configurationin intensively managed radiata pine plantations. Plant and Soil 71, 37-47.

Naschitz, S., Naor, A., Genish, S., Wolf, S. and Goldschmidt, E. E. 2010. Internal management of non-structural carbohydrate resources in apple leaves and branch wood under a broad range of sink and source manipulations. Tree Physiology 30, 715–727.

Nielsen, K. F. 1974. Roots and root temperatures. In: The plant root and its environment. Edited by E. W. Carson, The university press of Virginia. p. 293-322.

Nightingale, G. T. 1935. Effect of temperature on growth, anatomy and metabolism of Apple and Peach. *Botanical Gazette* 96(4), 581-639.

Osmont, K. S., Sibout, R. and Hardtke, C. S. 2007. Hidden Branches: Developments in Root System Architecture. Annual Review of Plant Biology 58, 93–113.

Palmer, J. W. 1988. Annual Dry Matter Production and Partitioning Over the First 5 Years of a Bed System of Crispin/M.27 Apple Trees at Four Spacings. Journal of Applied Ecology 25(2), 569-578.

Palmer, J. W. 1992. Effects of varying crop load on photosynthesis, dry matter production and partitioning of Crispin/M.27 apple trees. Tree Physiology 11, 19 – 33.

Paul, M.J. and Foyer, C.H. 2001. Sink regulation of photosynthesis. Journal of Experimental Botany 52(360), 1383-1400.

Peterson, C.A. and Enstone, D.E. 1996. Functions of passage cells in the endodermis and exodermis of roots. Physiologia Plantarum 97, 592-598.

Pregitzer, K. S., King, J. S., Burton, A. J. and Brown, S. E. 2000. Responses of tree fine roots to temperature. New Phytologist 147(1), 105-115.

Psarras, G. and Merwin, I.A. 2000. Water stress affects rhizosphere respiration rates and root morphology of young 'Mutsu' apple trees on M.9 and MM.111 rootstocks. Journal of American Society of Horticultural Science 125(5), 588–595.

Psarras, G., Mervin, I.A., Lakso, A.N. and Ray, J.A. 2000. Root growth phenology, Root Longevity, and Rhizosphere Respiration of Field Grown 'Mutsu' Apple Trees on 'Malling 9' Rootstock Journal of American Society of Horticultural Science 125(5), 596-602.

Rogers, W.S. 1939. Root studies VIII. Apple root growth in relation to rootstock, soil, seasonal and climatic factors. Journal of Horticultural Science 17, 99–130.

Rogers, W.S. and G.C. Head. 1969. Factors affecting the distribution and growth of roots of perennial woody species, p.111–148. In: W.J. Whittington (ed.). Root growth. Butterworths, United Kingdom.

Rom, C.R. 1996. Coordination of root and shoot growth: roots and rootstocks. In: K.M. Maib, P.K. Andrews, G.A. Lang and K. Mullinix (eds.), Tree Fruit Physiology: Growth and Development. Good Fruit Grower, Yakima, Washington, USA, p. 53-67.

Schechter, I., Elfving, D. C. and Proctor, J. T. A. 1991. Apple tree canopy development and photosynthesis as affected by rootstock. Canadian Journal of Botany 69(2), 295-300.

Schupp, J.R. and Ferree, D.C. 1990. Influence of time of root pruning on growth, mineral nutrition, net photosynthesis and transpiration of young apple trees. Scientae Horticulturae 42, 299-306.

Sharp, R. E., Poroyko, V., Hejlek, L. G., Spollen, W. G., Springer, G. K., Bohnert, H. J. and Nguyen, H. T. 2004. Root growth maintenance during water deficits: physiology to functional genomics. Journal of Experimental Botany 55 (407), 2343 – 2351.

Taiz, L. and Zeiger, E. 2010. Plant Physiology, 5th Edition, Sinauer Associates, Inc., p. 1-34, 468-472.

Terblanche, J. H. 1972. Seisoensopname en verspreiding van tien voedings elemente by jong appel bome gekweek in sand kulture. PhD tesis in Landbou, Universiteit van Stellenbosch.

Terblanche, J. H. 1986. Technical implications of the post-harvest physiology in the deciduous fruit. Deciduous Fruit Grower January, 23-27.

Tierney, G. L., Fahey, T. J., Groffman, P. M., Hardy, J. P., Fitzhugh, R. D., Driscoll, C. T. and Yavitt, J. B. 2003. Environmental control of fine root dynamics in a northern hardwood forest. Global Change Biology 9(5), 670-679.

Wang, Z. and Stutte, G. W. 1992. The role of carbohydrates in active osmotic adjustment in apple under water stress. Journal of American Society of Horticultural Science 117(5), 816-823.

Wells, C. E. and Eissenstat, D. M. 2001. Marked differences in survivorship among apple roots of different diameters. Ecology 82(3), 882-892.

Wells, C. E. and Eissenstat, D. M. 2003. Beyond the roots of young seedlings: the influence of age and order on fine root physiology. Journal of Plant Growth Regulators 21, 324-334.

White, P. J. 1998. Calcium channels in the plasma membrane of root cells. Annals of Botany 81(2), 173-183.

White, P. J. 2001. The pathways of calcium movement to the xylem. Journal of Experimental Botany 52(358), 891-899.

White, P. J. and Broadley, M. R. 2003. Calcium in plants. Annals of Botany 92(4), 487-511.

Withington, J. M. 2005. Thesis in Ecology (Ph. D). Fine root production and lifespan in eleven temperate tree species growing in a common garden in Poland. Pennsylvania State University.

Yao, S., Mervin, I. A. and Brown, M. G. 2009. Apple root growth, turnover, and distribution under different orchard groundcover management systems. Hortscience 44(1), 168–175.

Yao, S., Merwin, I.A. and Brown, M. G. 2006. Root dynamics of apple rootstocks in a replanted orchard. HortScience 41(5), 1149–1155.

Zimmerman, M. H., Brown, C. L. and Tyree, M. T. 1971. Trees: Structure and Function. Springer – Verlag, New York Inc, 51-57.

Paper 1

Quantifying white root growth dynamics of apple trees in the Western Cape region of South Africa.

Introduction

White root growth of deciduous trees, such as apple, are very important for nutrient uptake and phytohormone production, both of which are essential to tree performance and ultimately reproduction and fruit quality (Bouma et al. 2001; Ma et al, 2013). Newly produced fine roots, however, are short lived, have a high carbon cost and are not produced continuously throughout the season, especially for fruit bearing trees (Atkinson and Wilson, 1980; Cripps, 1970; Eissenstat et al., 2006; Yao et al., 2009). Quantifying the seasonal growth pattern of fine roots for a particular fruit type under specific growing conditions is therefore important to optimize fertilization scheduling and improve nutrient use efficiency, as well as for enhancing our understanding of carbon partitioning patterns (Eissenstat et al., 2006).

Generally, root growth in apples (*Malus domestica*) is reported as a phenological phenomenon with a bimodal pattern of activity (Atkinson and Wilson, 1980; Cripps, 1970; Fallahi, 1994; Rom, 1996), which tends to alternate with other competing sinks, such as shoot and fruit growth (Fumey et al., 2011; Head, 1967; Rom, 1996). According to these earlier reports on apple root dynamics, the first phase of fine root production either peaked around full bloom (Fallahi, 1994; Rom, 1996) or late spring (Atkinson and Wilson, 1980; Cripps, 1970; Head, 1967), before the occurrence of maximum shoot growth. A second growth cycle either commenced as shoot growth rates declined or succeeding fruit harvest, indicating some sink competition for assimilates (Atkinson and Wilson, 1980; Maggs, 1963; Palmer, 1992). Eissenstat (2006) reported a different root growth pattern for young, bearing ‘Gala’/M9 trees. He reported a strong root flush during bloom, followed by modest, but continuous root growth throughout the remainder of the season, with no root flush after harvest in the first year. The following spring, no indication of root activity was observed during bloom, instead, root growth increased steadily during summer in the second year, declined near harvest time and was followed by a strong root flush after harvest. Psarras et al. (2000) reported one main peak of root growth for ‘Mutsu’/M9, which partially coincided with periods of shoot and fruit growth for two consecutive years. Similar results were later reported by Yao et al. (2006), where root growth

peaked between late May and July (November and January - Southern hemisphere), coinciding with the main phase of shoot and fruitlet growth. These studies suggested that sink competition, as a factor controlling root growth patterns, varies in magnitude between orchards due to differences in degree of influence from genotype, endogenous physiological processes, environmental conditions and cultural practices (Joslin et al., 2001; Psarras et al., 2000; Rogers and Head, 1969). This complex interaction of internal and external factors could therefore potentially lead to high variation between orchards (Rogers and Head, 1969). This was evident in recent minirhizotron (MR) data from Psarras et al. (2000), which revealed a contrasting root growth pattern compared to most of the earlier reports.

The slow advance in root research is mainly due to technical difficulties resulting from poor accessibility (Mancuso, 2012). Information on root growth dynamics and architecture in relation to environmental conditions is therefore limited relative to knowledge on aerial growth (Fageria, 2013). More contemporary research using MR technology, which allows proper replication, reveals a greater variety of fine root growth patterns than reported in earlier, destructive root studies, where well-replicated experiments were not possible (Eissenstat et al., 2006; Li et al., 2003; Ma et al., 2013; Psarras, 2000; Yao et al., 2006, 2009). The MR method provides a continuous, non-destructive observation of white root production as part of a replicated experimental design under field conditions and is reliable for the purpose of studying long term root dynamics (Abrisqueta et al., 2008; Eissenstat et al., 2006; Fukuzawa et al., 2012; Gluszek et al., 2013; Vamerali et al., 2012; Withington, 2005). Limitations of MR applications include investigation of root architectural aspects due to the high variability of root distribution and the relatively small sampling surface of the tube (Böhm, 1979; Yao et al., 2009). Nevertheless, MR technology offers a greater potential for replication than the more expensive, static rhizotron chambers and a potential for more continuous undisturbed observations than destructive sampling techniques used by earlier researchers (Eissenstat et al., 2006; Gluszek et al., 2013).

As most reports on the seasonal patterns of apple root growth originated in temperate Northern hemisphere regions with severe winters (Atkinson and Wilson, 1980; Eissenstat et al. 2006; Psarras et al., 2000; Rogers and Head, 1969) they may differ from those occurring in warmer Southern hemisphere regions like the Western Cape, with a mild winter climate, and thus may not be suitable for direct and purposeful interpretation under local conditions. In this paper, we described root growth dynamics of i) young, non-bearing ‘Corder Gala’ apple trees in a sandy

soil, ii) mature, bearing 'Golden Delicious' apple trees in clay soil, iii) mature, bearing 'Cripps Pink' apple trees in a sandy soil and iv) young bearing 'Fuji' apple trees in a clay soil, as four examples of possible growth patterns of trees on vigorous rootstocks in the Elgin-Vyeboom area. This information was then related to tree phenology to indicate i) whether root growth dynamics under local conditions confirm similar reports published abroad and ii), whether young and mature, bearing trees show similar growth patterns during the season.

Materials and Methods

Plant Material & Experimental sites

Young, non-bearing 'Corder Gala' orchard

'Corder Gala' apple trees on M7 rootstock, planted during the spring of 2012, on a commercial farm ('Vyeboom Plaas') in the Vyeboom area (S 34° 05'19.8" E 019° 05'24.7") were used as one experimental site. The site is characterized by a sandy loam soil which remains naturally moist throughout most of the year at deeper soil depths (below 0.70 m). Tree spacing was 2 m x 4 m. Irrigation by micro-jets was scheduled by the farm to maintain soil water close to field capacity. During the second season (2013/14) trees were trained to a solax system.

This trial was performed simultaneously on the same site with a second trial evaluating cover crops which will not be discussed in this paper. The cover crops were only sown during the second season of our trial and were thus not regarded as a treatment that influenced the current root observations. Five replicates were chosen randomly, each plot comprising two experimental trees, of which an MR tube was installed next to one tree. Tree rows were clean cultivated using herbicides.

Mature, bearing 'Golden Delicious' orchard

This mature bearing orchard consisted of 'Golden Delicious' trees on M793 rootstock, which was established in 2007. The orchard was situated on the commercial farm, Applegarth, located in Grabouw (S 34° 08'10.2" E 019° 02'04.4"). The soil is characterized as a heavy clay loam, with a 50 % stone fraction and clay horizon occurring in between depths of 0.6 and 1.2 m (no

soil classification was available). Tree spacing was 2 m x 4.5 m. Nine replicates were randomly selected, where each plot was represented by two trees. A single MR tube was installed at one tree per plot. These plots formed part of another experiment which is discussed in Paper 3. For the purpose of this discussion, one replicate represents root growth of one tree. Irrigation was scheduled by the farm management on an ad hoc basis, using evapotranspiration data, by means of micro-jets. Tree rows were mowed and sprayed with herbicides once per year during summer, allowing for weed and grass growth throughout most of the year.

Mature, bearing 'Cripps Pink' orchard

The 'Cripps Pink' trees on M793 rootstock were planted in 2005 on a well drained sandy soil. The orchard is located on the commercial farm Somersfontein in Grabouw. Tree spacing was 2 m x 4.5 m. Two replicates (trees) were randomly selected, with a MR tube installed at both trees. Trees were irrigated using micro-jets and scheduling by the farm management was based on evapotranspiration data. Tree rows were clean cultivated using herbicides.

Young, bearing 'Fuji' orchard

The young 'Fuji' orchard was established in 2009 on M793 rootstock on a clay loam soil. The orchard was also located on the commercial farm Applegarth in Grabouw. Tree spacing was 2 m x 4.5 m. The first season of cropping was 2012/2013. Two replicates, each representing a single tree was randomly selected for the installation of one MR tube per tree. Irrigation through micro-jets was scheduled by the farm management using evapotranspiration data. Tree rows were clean cultivated using herbicides.

Measurements

Root growth dynamics

Root activity was monitored using MR tubes and a root scanner (CI-600, CID Bioscience, Inc, Camas, WA USA). Acrylic butyrate tubes with a length of 1.05 m were installed parallel to the tree row, 40 cm from the tree base at an angle of approximately 45° using a spiral auger (Linsenmeier et al., 2010; Vamerali et al., 2012) during April 2013. Image collection

commenced shortly after installation, as white root emergence was observed (through excavation) from early April 2013, between 0 - 30 cm soil depth, following harvest.

Root scanning with the CI-600 Root Scanner was performed regularly from 21 May 2013 to November 2014. During peak root growth phases, weekly to bi-weekly scans were performed whereas longer, monthly intervals were implemented during periods with less root growth. Periods with high white root activity was clearly noticable compared to periods with low activity. From August until November 2013 no scanning with the Root Scanner was possible due to technical and logistical difficulties. Root activity during this period was determined by manually digging 30 cm deep soil profiles 30 cm from the trunk for 3-5 trees on a monthly basis for some visual indication of root activity in the upper soil layers.

In order to construct a complete image of the total tube length (approx. 90 cm) and soil depth (approx. 60 cm) covered, four scans representing four 'windows' were recorded at each collection date. A 21.59 × 19.56 cm colour image of the soil and roots was obtained for each window. Root numbers per image (window) were quantified by physically counting the number of white roots (Fig. 1). The total number of white roots were calculated for each tube and averages were presented per site for the two seasons.

Young apple trees during establishment have a lower root length density (RLD) compared to mature trees (Hudges and Gandar, 1993; Yao et al., 2006). Therefore, additional 50 x 50 cm deep soil profiles were made at the young non-bearing orchard on dates where no white roots were detected in the MR windows, to determine if white roots were active further away from the tree base than the MR tubes. Five individual profiles of different non-experimental trees were randomly investigated at each MR date (between 13 February and 23 June 2014) in close proximity to the MR trees.

Tree phenology

The phenological progression for the 'Golden Delicious' orchard was documented to relate this to the root growth pattern. Phenological development: bud break, full bloom, fruit set, maximum shoot growth, termination of short and long shoot growth, maximum fruit growth, harvest, onset of leaf drop; 50 % leaf drop and 100% leaf drop, were all recorded.

Root distribution

Root distribution was quantified through root count observations by utilizing a profile study similar to the method described by Böhm (1979). Observations for the mature bearing 'Golden Delicious' trees were performed on 5 June 2013 and 12 May 2014 and, for the young non-bearing 'Corder Gala' trees, on 14 May 2013 and 19 May 2014. Due to this being a destructive method, only three replicates per site, per year were quantified. A vertical soil surface area of 1 m^2 was exposed next to three trees, 30 cm from the trunk. Exposed roots in the profile were spray-painted white for better visibility, categorized according to four diameter categories (<2 mm, 2-5 mm, 5-10 mm and >10 mm) and counted according to the methods of Böhm (1979). The counting process was simplified using a metal grid dividing the 1 m^2 profile into 10 cm^2 blocks.

Results

Root growth dynamics – Mature bearing 'Golden Delicious' trees

The first MR scan occurred on 21 May 2013, revealing white root growth (Fig. 1 and 2) in all 9 tubes at this site. However, white root tips were already noticed (by means of excavation) two weeks after harvest (13 March 2013) in the top 30 cm soil. According to MR images, a period of strong root production led to a peak in white root occupancy in late June 2013 (Fig. 3). A variation between trees in terms of root activity was evident with four replicates producing a strong peak, two trees only revealing intermediate root production and three trees producing low amounts of white roots (Data not shown). Overall, root activity declined towards the end of July 2013. Evidence for some root activity during late winter (August) and early spring (September) were deducted from differences in root occupancy between 31 July and 3 October as the MR equipment was not available during late winter and early spring of 2013. Root images from 3 October 2013 reveal an increased occupancy of brown roots for some replicates indicating that root growth, followed by root browning, occurred during winter. Modest root activity continued during bloom, fruit set and shoot growth and started to decline at the end of January 2014 towards harvest (4 March 2014). Very low rates of root growth occurred during February and March 2014, with the exception of a short flush observed in one tree. White root occupancy was therefore negligible during the month before harvest. Root production returned to moderate rates about 2 weeks after harvest and remained so until early

May, where white root numbers started to increase, and peaked in early June 2014. The post-harvest root growth trend therefore shows some variation in timing between 2013 and 2014 with the latter being earlier. Total white root numbers continued to increase substantially during June 2014, slowly declining during July and August (similar to 2013), therefore confirming active root growth throughout winter. Very low activity was observed during bud break (mid September), which increased slightly after full bloom (mid October), followed by rapid production of white roots during fruit set. White root activity remained high during active shoot growth in late November 2014. This strong summer peak in root production was followed by rapid root browning, which substantially decreased white root numbers over a 2 week period coinciding, with increasing fruit growth rates.

Root growth dynamics – Mature bearing 'Cripps Pink'

The most pronounced flush of root production in this orchard commenced in May (2013 and 2014) less than a month after harvest (Fig 4). A rapid increase in white roots occurred in mid-June of both seasons (2013, 2014). In 2014, however, white root numbers declined earlier than in 2013. From August 2014 until November 2014 root growth remained negligible. A substantially smaller summer root flush (compared to the winter flushes) occurred during early December 2014. Negligible new root production occurred from late December until May 2014.

Root growth dynamics – Young, bearing 'Fuji'

The 2012/13 season was the first cropping year for this orchard. Root growth started to increase approximately 6 weeks following the first harvest (late March) (Fig 5). White root counts increased from May until mid-June 2013 and slowly decreased during the remainder of the winter. White root activity was low during spring 2013. Root observations during summer 2013 were not possible due to technical difficulties with the MR scanner. A low level of root emergence occurred in February and March 2014, preceding harvest (3 April 2014), after which a rapid increase in white root numbers occurred from May, which peaked in June and decreased slowly throughout winter. The winter root flush of 2014 produced more than double the white roots, than in winter 2013. A few white roots were still present during early spring (5 September 2014). A very small summer flush then occurred in early November 2014, which declined by December 2014.

Root growth dynamics – Young non-bearing 'Corder Gala'

New root growth was observed with the first MR scan on 21 May 2013. White root occupancy subsequently declined to very low numbers towards the end of July 2013 (Fig. 6). The first root scan in spring (3 October 2013) indicate that root numbers remained low during winter. Total white roots drastically increased from late November, showing two successive peaks interrupted by a sharp decline in roots during mid-January 2014. Most white roots then proceeded to mature with no new root production evident, according to the MR scans, as total white root counts decreased during February and March 2014. White root occupancy around the tubes remained negligible throughout autumn and winter (April to June 2014). However, white roots occupied a position further away from the tree base (> 50 cm) compared to the positioning of the MR tubes (30 cm from tree base), determined by means of excavation on the MR dates during late summer to early autumn (between 13 February and 25 April 2014) (data not shown). White roots at the excavation distance (> 50 cm from tree base) became less noticeable from 19 May to 23 June 2014. Therefore, neither the MR data nor the excavation data revealed significant root activity during winter 2014. Root production in this young non-bearing apple orchard therefore shows potential for continuous growth from early spring until leaf drop in autumn, revealed by combining results from MR images and the excavation data.

Tree Phenology – Mature 'Golden Delicious' orchard

The onset of bud break in September 2013 was followed by full bloom in mid-October 2013 (Fig. 3) Growth of short shoots terminated late in December 2013, following a two month growth phase that commenced in late October 2013. Long shoots continued growth until late in January 2014. Fruit growth occurred from fruit set in late October, until harvest on 4 March 2014. The onset of leaf drop was noted in late April 2014 with 50 % leaf drop during late May 2014. Dormancy was completed with signs of bud break in the second week of September 2014, slightly earlier than in 2013. Consequently, full bloom and fruit set was a week earlier in 2014 compared to 2013, although shoot growth patterns were similar. In 2014 fruits were harvested on 4 March, almost 2 weeks earlier than in 2013. Leaf drop, however, seemed to occur at a similarly slow rate than in 2013, where functional leaves remained at least until late in May.

Root Distribution – Young non-bearing 'Corder Gala'

Roots were observed to a depth of 60 and 70 cm in 2013 and 2014 respectively (Fig 7 and 8). The distribution of the fine root category (< 2 mm) seemed to change from 2013 to 2014 as fewer fine roots were observed at 30 cm from the tree base in 2014 compared to the previous year. The average amount of fine roots per season decreased from 284 in 2013 to 152 in 2014 (Appendix, Fig. 1 and 2).

Root distribution - Mature bearing 'Golden Delicious'

The main difference between the root distribution studies of 2013 and 2014 was rooting depth. In 2013 roots were more evenly distributed and occupied the soil to a greater depth (100 cm), whereas roots in 2014 were sparsely distributed below 60 cm (Fig 9 and 10). However, the shallower (60 cm) clay layer (which was not present at the 2013 root distribution study) most likely resulted in the decreased number of roots growing below 60 cm soil depth for the trees chosen in 2014. The average fine root counts of the three profiles for the top 60 cm were 449 (2013) and 518 (2014), indicating a slight increase from 2013 to 2014 (Appendix, Fig. 3 and 4). However, taking the whole 100 cm^2 profile into account, the number of fine root decreased from 711 (2013) to 574 (2014) over the two years of the study.

Discussion

The root production trend for the bearing 'Golden Delicious', 'Cripps Pink' and 'Fuji' orchards resemble a bimodal seasonal pattern, with a peak in both summer and autumn/winter. The occurrence of a summer root growth peak agrees with some earlier reports on apple root growth dynamics (Atkinson and Wilson, 1980; Cripps, 1970; Rogers and Head, 1969), although the timing, intensity and duration of the peak disagrees with others (Eissenstat et al., 2006; Fallahi, 1994; Psarras et al., 2000; Rom, 1996). The summer flush for all three bearing orchards in this study occurred during November/December, the timing of which seems to be similar but perhaps slightly later than reports by Atkinson and Wilson (1980) and Head (1967) who observed a peak around May, and slightly earlier than Yao et al. (2006) with a peak between May and July in the Northern hemisphere, as well as that observed by Cripps (1970), between November and January in the Southern hemisphere. The summer peak for the 'Cripps Pink' and 'Fuji' orchards, however, were substantially smaller compared to their respective post-

harvest peaks which agrees with the smaller peak in 'Worcester'/M9 reported by Atkinson and Wilson (1980). Furthermore, white root production was generally low ('Golden Delicious') or negligible ('Cripps Pink' and 'Fuji') when the fruit were growing rapidly prior to harvest. This is fairly commonly reported for apple root growth dynamics, as high fruit growth rates may compete with roots for available photosynthates (Atkinson and Wilson, 1980; Eissenstat et al., 2006; Palmer, 1992; Yao et al., 2006). However, Psarras et al. (2000) reported a single peak in root production, which overlapped with high shoot and fruit growth rates for two consecutive years in 'Mutsu' apple trees, demonstrating the potential variability associated with root growth patterns of woody perennials (Rogers and Head, 1969).

All three bearing orchards in this study consistently produced a strong post-harvest root growth peak in June, which started shortly after fruit harvest, showing high white root activity during leaf drop (early winter), followed by a decrease in root numbers during late winter. Reports on root production during tree dormancy are not common, as most studies have reported an absence of root growth during autumn/winter for apple (Atkinson and Wilson, 1980; Cripps, 1970; Psarras et al., 2000; Yao et al., 2006), as well as for deciduous forests (Burke and Raynal, 1994; Côté et al., 1998; Kuhns et al., 1985; Tierney et al., 2003; Withington, 2005). The lack of root growth during winter dormancy in the Northern hemisphere regions can be attributed to the low soil temperature conditions (Psarras et al., 2000; Rogers and Head, 1969). However, under adequate soil temperature conditions (9°C) in the Southern hemisphere, Cripps (1970) also found root growth in apple to be uncommon during tree dormancy, suggesting the lack of current photosynthates to be the cause. Furthermore, Eissenstat et al. (2006) found that post-harvest root growth was inconsistent between years for 'Gala'/M9 and never observed a root growth flush in autumn for 'Golden Delicious'/M9. Research on apple root dynamics therefore shows that root growth patterns can vary greatly between consecutive years, in addition to the variance found between different orchards. The complex interaction between environmental conditions, cultural practices, tree age, cropping intensity and rootstock type is most likely responsible for the high potential variability observed for the onset, magnitude and duration of a root growth flush in fruit trees (Atkinson and Wilson, 1980; Ma et al., 2013; Rogers and Head, 1969).

Root growth peaks for the young, non-bearing trees were not as clear and may be attributed to the continuous root growth potential of establishing apple trees (Cripps, 1970) and, in part, due

to their low root length density (RDL) away from the tree trunk (Hughes and Gandar, 1993; Yao et al., 2006). Low RLD consequently results in low root counts using the MR method due to lower root interception at the MR tube window (Yao et al., 2006), as more pioneer roots, which become structural and grow further away from the tree base, are produced by young trees for the purpose of root system establishment (Espeleta and Eissenstat, 1998; Hudges and Gandar, 1993; Polverigiani et al., 2011; Rogers and Head, 1969). This was also evident in the root distribution study in the young orchard as the number of fine roots, at a position similar to the MR (from the tree trunk), decreased from 2013 to 2014. Active white root tips were, however, observed (determined by personal excavation) at a position (> 50 cm from tree base) further than the MR tube from the tree base in 2014 (esspecially February until April), while the MR images showed negligible numbers of white roots. Root distribution in apple trees younger than 4 years shows high variability compared to older trees, which have a more even root distribution (Hudges and Gandar, 1993).

Nevertheless, in agreement with Cripps (1970), root activity seemed more prominent during the growing season (from bud break until leaf drop), compared to the negligible or inconsistent root activity during winter (tree dormancy). Although high white root numbers were observed during the winter of 2013, root growth may have been stimulated by a root pruning effect during tube installation (Côté et al., 1998). Negligible root activity was observed the following winter (2014), either with MR or the root profile studies.

The use of MR technology seems to be best suited for studying long term trends in root production under field conditions compared to other root growth monitoring methods such as sequential soil coring and root ingrowth methods (Fukuzawa et al., 2012; Hendricks et al., 2006). Furthermore, the MR method produces more reliable results than the large static rhizotrons used in earlier studies (Atkinson and Wilson, 1980), as it is better suited for a properly replicated experimental design (Eissenstat et al., 2006; Milchunas, 2012). However, similar to all the root growth quantification methods, MR data may also be compromised by its associated assumptions and sampling errors (Hendricks et al., 2006). Variation may also arise from different tube installation, image collection and data processing methods (Milchunas, 2012). The probability of intercepting roots may also be influenced by insertion angle of the tube, as well as root distribution. As observed in our study, young trees have a significantly lower RDL resulting in lower root counts due to low root interception at the tube compared to mature trees (Yao et al., 2006). The root distribution of young and mature trees

therefore differ, which is also evident in their different root distribution results (Fig. 7, 8, 9 and 10). Although an insertion angle of 20 – 45° to the surface improves the chances for root interception by the tube (Milchunas, 2012), low-density root systems, such as the young trees in this study, may require a greater amount of tubes per replicate.

Conclusion

In the Elgin – Vyeboom area of South Africa, the dynamics of apple white root growth of bearing trees differ from the dynamics of young, non-bearing trees. Root growth patterns of bearing trees from three different scions ('Golden Delicious', 'Cripps Pink' and 'Fuji') on the same vigorous rootstock (M793) on two contrasting soil types (sandy and heavy clay) in the same climatic region, seem to follow a bimodal pattern, with the first flush in summer and the second, larger flush after fruit harvest in autumn/winter. This seems to be a general trend for bearing trees, with the mentioned factors playing a secondary role in the onset and finish of the flushes. Although our root growth data corresponds to a bimodal pattern, the timing of the autumn/winter root flush in this study is temporally unique and differs from most literature reporting on apple root growth dynamics showing no active white root growth during winter. These findings may therefore have unique implications for management practices regarding nutrition and perhaps pruning, due to the significant effect of root growth on the carbon balance of the tree.

Root growth activity was less predictable for the young, non-bearing trees due to its dynamic architecture and lower RLD. This results in substantially lower white root numbers at the MR window compared to bearing trees, making it difficult to identify peaks. For young establishing trees, additional MR tubes (installed at different distances from the tree trunk) could therefore improve the validity of the data by compensating for the low RLD. Further studies involving the quantification of root growth in young trees should therefore take root architecture into account when planning the number and positioning of MR tubes for each tree. On the other hand, root growth of young apple trees (unlike mature trees) may show uninterrupted activity (with less pronounced peaks) throughout the season. This theory may be valid in such an orchard when taking into account both the MR and excavation data, as well as the developmental pattern of young root systems.

References

Abrisqueta, J.M., Mounzer, O., Alvarez, S., Conejero, W., García-Orellana, Y., Tapia, L.M., Vera, J., Abrisqueta, I. and Ruiz-Sánchez, M.C. 2008. Root dynamics of peach trees submitted to partial rootzone drying and continuous deficit irrigation. Agricultural water management, *95*(8), 959-967.

Atkinson, D. and Wilson, S.A. 1980. The growth and distribution of fruit tree roots: some consequences for nutrient uptake. (eds.) Atkinson, D., Jackson, J. E., Sharples, R. O. and Waller, W. M. *Mineral nutrition of fruit trees*. Butterworths publishers 1980, 137-150.

Böhm, H. 1979. Methods of Studying Root Systems. Springer Verlag, New York, 48-60.

Bouma, T. J., Yanai, R. D., Elkin, A. D., Hartmond, U., Flores- Alva, D. E. and Eissenstat, D. M. 2001. Estimating age- dependent costs and benefits of roots with contrasting life span: comparing apples and oranges. *New Phytologist*, *150*(3), 685-695.

Burke, M. K. and Raynal, D. J. 1994. Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. *Plant and Soil*, *162*(1), 135-146.

Côté, B., Hendershot, W. H., Fyles, J. W., Roy, A. G., Bradley, R., Biron, P. M.and Courchesne, F. 1998. The phenology of fine root growth in a maple-dominated ecosystem: relationships with some soil properties. *Plant and Soil*, *201*(1), 59-69.

Cripps, J. E. L. 1970. A seasonal pattern of apple root growth in Australia. *Journal of Horticultural Science* 45, 153-161.

Eissenstat, D. M., Lakso, A. N. Neilsen, D., Neilsen, G. H. and Smart, D. R. 2006. Seasonal patterns of root growth in relation to shoot phenology in Grape and Apple. *Acta Horticulturae* 721, 21 -26.

Espeleta, J. F. and Eissenstat, D. M. 1998. Responses of citrus fine roots to localized soil drying: a comparison of seedlings with adult fruiting trees. *Tree Physiology*, *18*(2), 113-119.

Fageria, N. K. 2013. The role of plant roots in crop production. Taylor and Francis group, LLC, p. 8-14, 20-33, 159,185-191, 323.

Fallahi, E. 1994. Root physiology, development and mineral uptake. p.19-30. In: A.B. Peterson and R.G. Stevens (eds.), Tree Fruit Nutrition: A Comprehensive Manual of Deciduous Tree Fruit Needs. Good Fruit Grower, Yakima, Washington, USA.

Fukuzawa, K., Dannoura, M. and Shibata, H. 2012. Fine root dynamics and root respiration. Measuring roots, Mancuso, S (ed.), Springer- Verlag Berlin Heidelberg 2012, Chapter 15, p. 341-356.

Fumey, D., Lauri, P., Guedon, Y., Godin, C. and Costes, E. 2011. How young trees cope with removal of whole parts of shoots: An analysis of local and distant responses to pruning in 1-year-old apple (*Malus*× *Domestica*; Rosaceae) trees. *American Journal of Botany* 98 (11), 1737-1751.

Gluszek, S., Paszt, L. S., Sumorok, B., Derkowska, E. and Kozera, R. 2013. Application of the minirhizotron technique to studying the roots of fruit plants. *Advances in Science and Technology Research Journal* 7(18), 45–53.

Head, G.C. 1967. Effects of seasonal changes in shoot growth on the amount of unsuberized root on apple and plum trees. *Journal of Horticultural Science* 42, 69-180.

Hendricks, J. J., Hendrick, R. L., Wilson, C. A., Mitchell, R. J., Pecot, S. D. and Guo, D. 2006. Assessing the patterns and controls of fine root dynamics: an empirical test and methodological review. *Journal of Ecology 94*(1), 40-57.

Hughes, K. A. and Gandar, P. W. 1993. Length densities, occupancies and weights of apple root systems. *Plant and Soil* 148, 211-221.

Joslin, J. D., Wolfe, M. H. and Hanson, P. J. 2001. Factors controlling the timing of root elongation intensity in a mature upland oak stand. *Plant and Soil 228*(2), 201-212.

Kuhns, M. R., Garrett, H. E., Teskey, R. O. and Hinckley, T. M. 1985. Root growth of black walnut trees related to soil temperature, soil water potential, and leaf water potential. *Forest Science 31*(3), 617-629.

Li, K.T., Lakso, A.N., Piccioni, R. and Robinson, T., 2003. Summer pruning effects on fruit size, fruit quality, return bloom and fine root survival in apple trees. *Journal of Horticultural Science & Biotechnology 78*(6), 755-761.

Linsenmeier, A., Lehnart, R., Löhnertz, O. and Michel, H. 2010. Investigation of grapevine root distribution by in situ minirhizotron observation. *VITIS-Journal of Grapevine Research 49*(1), 1-6.

Ma, L., Hou, C. W., Zhang, X. Z. Li, H. L., Han, De G., Wang, Y. and Han, Z. H. 2013. Seasonal growth and spatial distribution of Apple tree roots on different rootstocks or interstems. *Journal of American Society of Horticultural Science* 138(2), 79–87.

Maggs, D. H. 1963. The reduction in growth of apple trees brought about by fruiting. *Journal of Horticultural Science* 38(2), 119-128.

Mancuso, S. 2012. Measuring roots, An updated approach. Springer-Verlag Berlin Heidelberg. Preface, pp. v-vi.

Milchunas, D. G. 2012. Biases and errors associated with different root production methods and their effects on field estimates of belowground net primary production. In: Mancuso, S. (eds.) Measuring roots, an updated approach. Springer-Verlag Berlin Heidelberg, p. 303-335.

Palmer, J. W. 1992. Effects of varying crop load on photosynthesis, dry matter production and partitioning of Crispin/M.27 apple trees. *Tree Physiology* 11, 19 – 33.

Polverigiani, S., McCormack, M. L., Mueller, C. W. and Eissenstat, D. M. 2011. Growth and physiology of olive pioneer and fibrous roots exposed to soil moisture deficits. *Tree Physiology 31*(11), 1228-1237.

Psarras, G., Merwin, I. A., Lakso, A. N. and Ray, J. A. 2000. Root growth phenology, root longevity, and rhizosphere respiration of field grown 'Mutsu' apple trees on 'Malling 9' rootstock. *Journal of the American Society for Horticultural Science 125*(5), 596-602.

Rogers, W.S. and G.C. Head. 1969. Factors affecting the distribution and growth of roots of perennial woody species, p. 111–148. In: W.J. Whittington (ed.). Root growth. Butterworths, United Kingdom.

Rom, C.R. 1996. Coordination of root and shoot growth: roots and rootstocks. p.53-67. In: K.M. Maib, P.K. Andrews, G.A. Lang and K. Mullinix (eds.), Tree Fruit Physiology: Growth and Development. Good Fruit Grower, Yakima, Washington, USA.

Tierney, G. L., Fahey, T. J., Groffman, P. M., Hardy, J. P., Fitzhugh, R. D., Driscoll, C. T. and Yavitt, J. B. 2003. Environmental control of fine root dynamics in a northern hardwood forest. *Global Change Biology 9*(5), 670-679.

Vamerali, T., Bandiera, M. and Mosca, G. 2012. In: Minirhizotrons in modern root studies. Measuring roots, Mancuso, S (ed.), Springer- Verlag Berlin Heidelberg 2012, p. 341-356.

Withington, J. M. 2005. Ph. D. Thesis in Ecology. Fine root production and lifespan in eleven temperate tree species growing in a common garden in Poland. Pennsylvania State University.

Yao, S., Merwin, I. A. and Brown, M. G. 2006. Root dynamics of apple rootstocks in a replanted orchard. *HortScience 41*(5), 1149-1155.

Yao, S., Merwin, I. A. and Brown, M. G. 2009. Apple root growth, turnover, and distribution under different orchard groundcover management systems. *HortScience 44*(1), 168-175.

Figure 1: A single MR image representing one of the four sections (windows) of the tube with a full size of 21.6 cm (width) X 19.6 cm (length). Marked roots indicate examples of white apple roots used to quantify root number in this study.

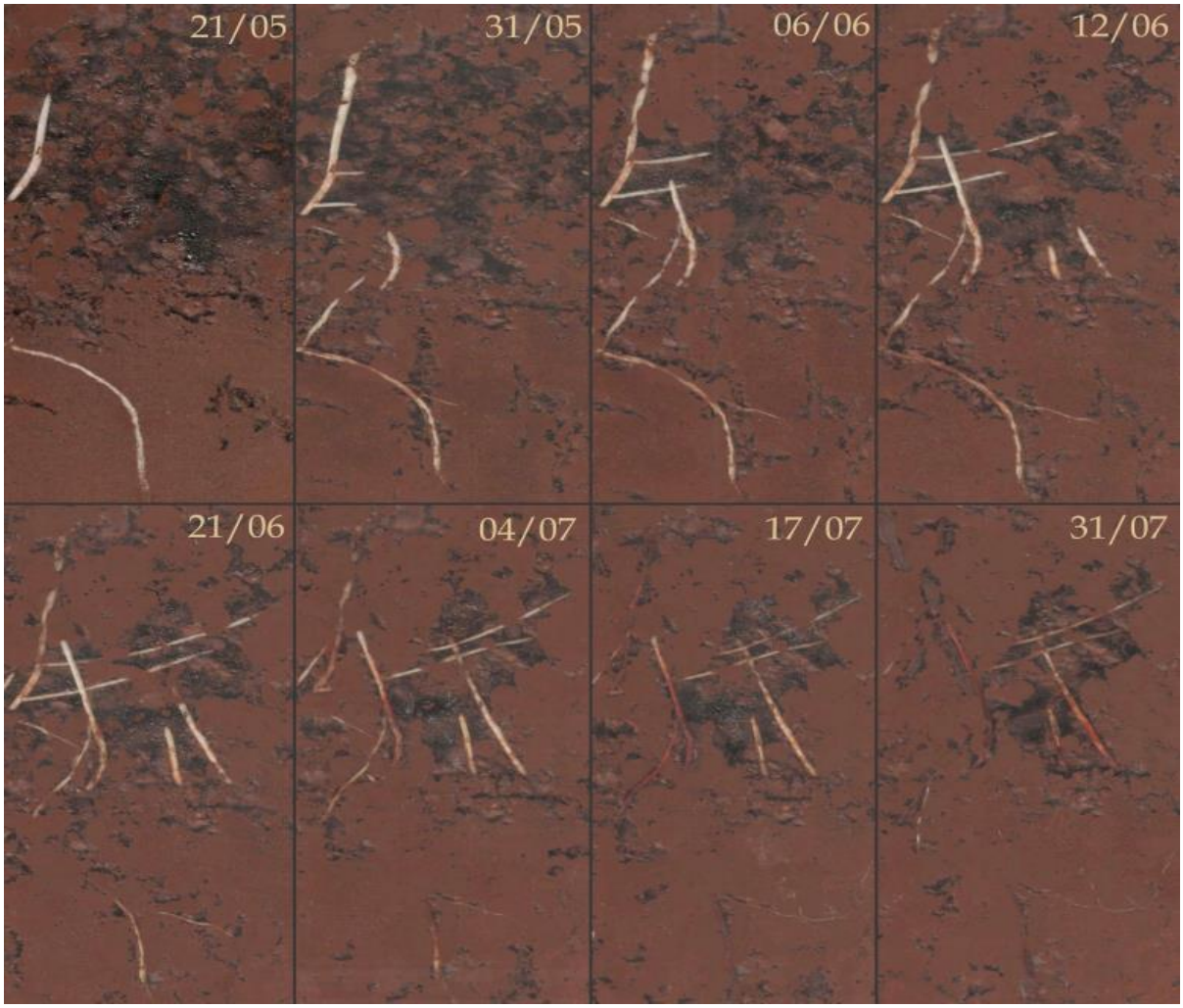

Figure 2: A section of a single window in sequence from 21 May 2013 until 31 July 2013 indicating the emergence, development and maturation of newly produced roots of 'Golden Delicious'/M793 in a clay loam soil.

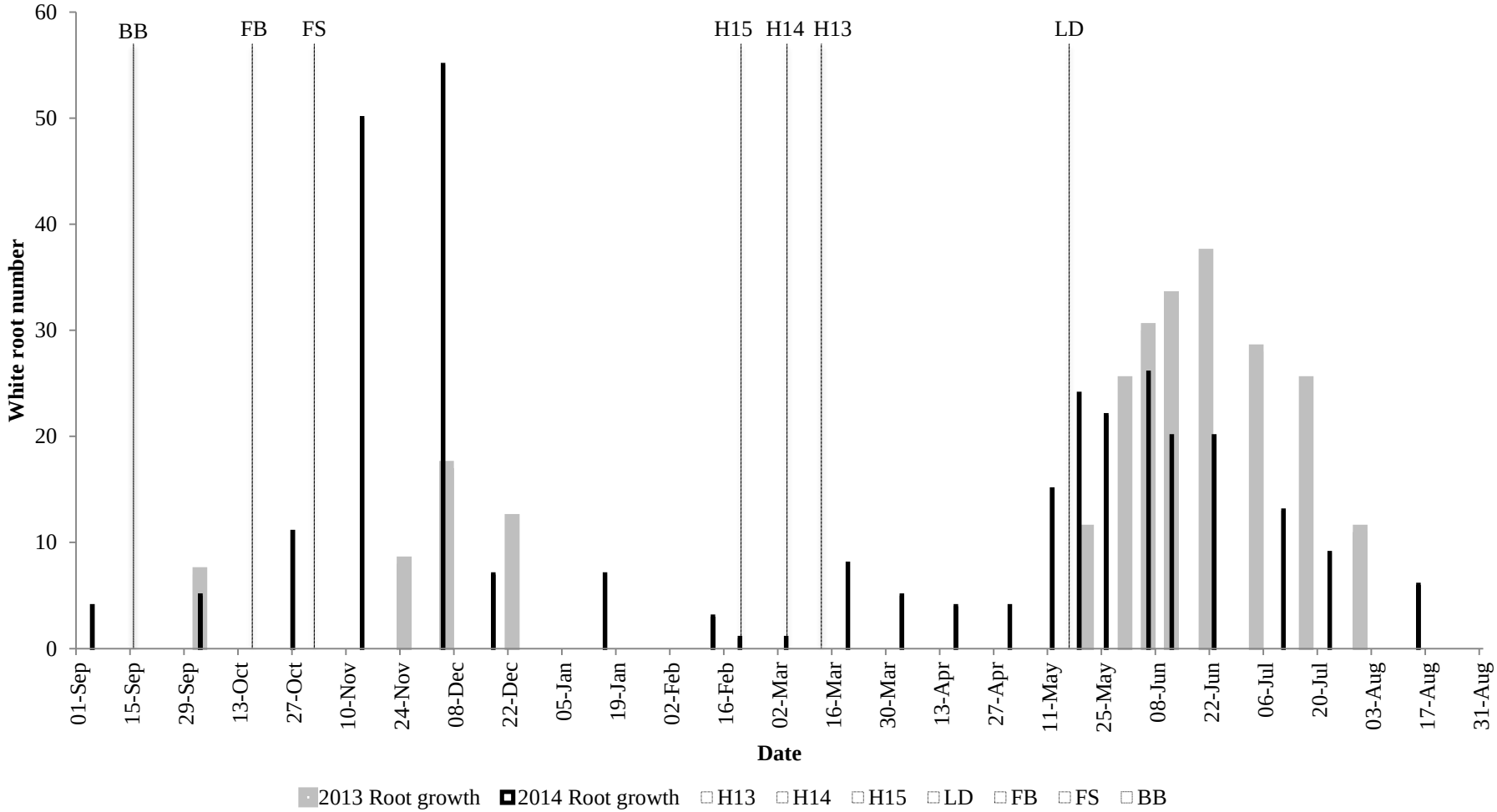

Fig 3. The seasonal change in average white root numbers (determined by minirhizotron images) in relation to phenological events, including bud break (BB), full bloom (FB), fruit set (FS), fruit harvest – 2013 (H13), 2014 (H14), 2015 (H15) and 50 % leaf drop (LD) for two consecutive seasons from 1 May 2013 until December 2014 for mature bearing 'Golden Delicious'/M793.

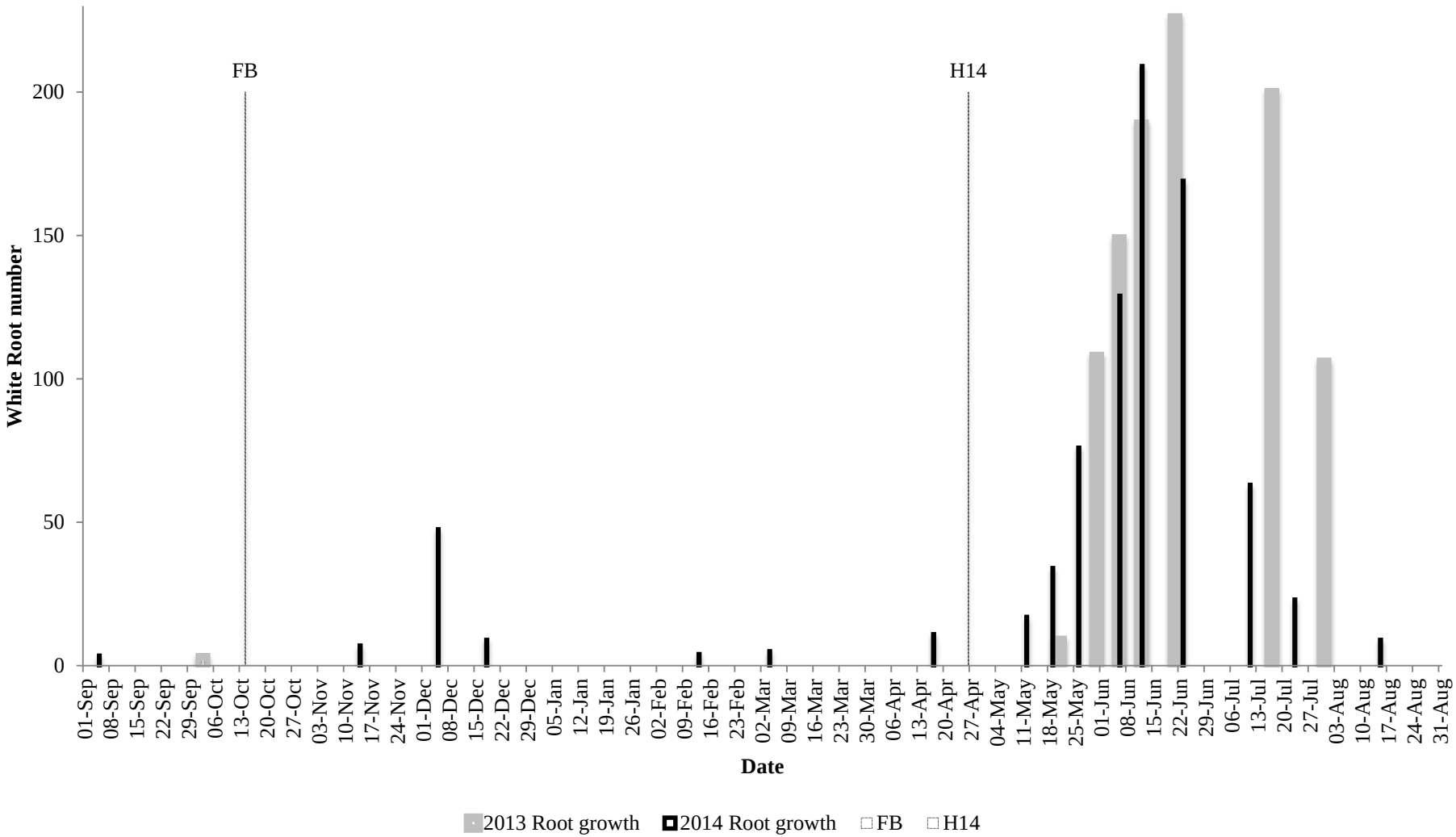

Fig 4. The seasonal change in average white root numbers (determined by minirhizotron images) in relation to phenological events, including full bloom (FB) and fruit harvest 2014 (H14) quantified from May 2013 until December 2014 for mature, bearing 'Cripps Pink'/M793.

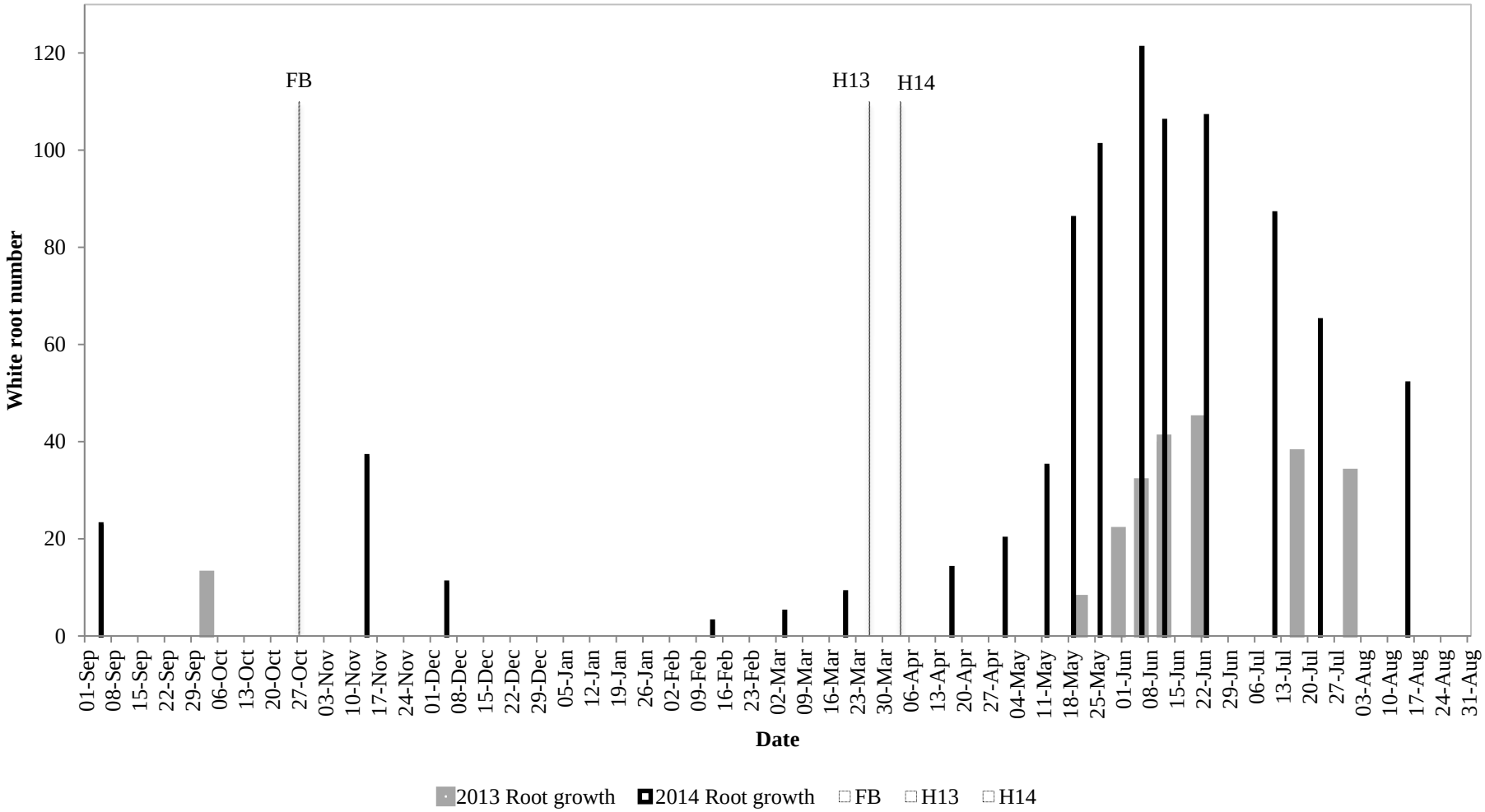

Fig 5. The seasonal change in average white root numbers (determined by minirhizotron images) in relation to phenological events, including full bloom (FB) and fruit harvest – 2013 (H13) and 2014 (H14) quantified from May 2013 until December 2014 for bearing 'Fuji'/M793.

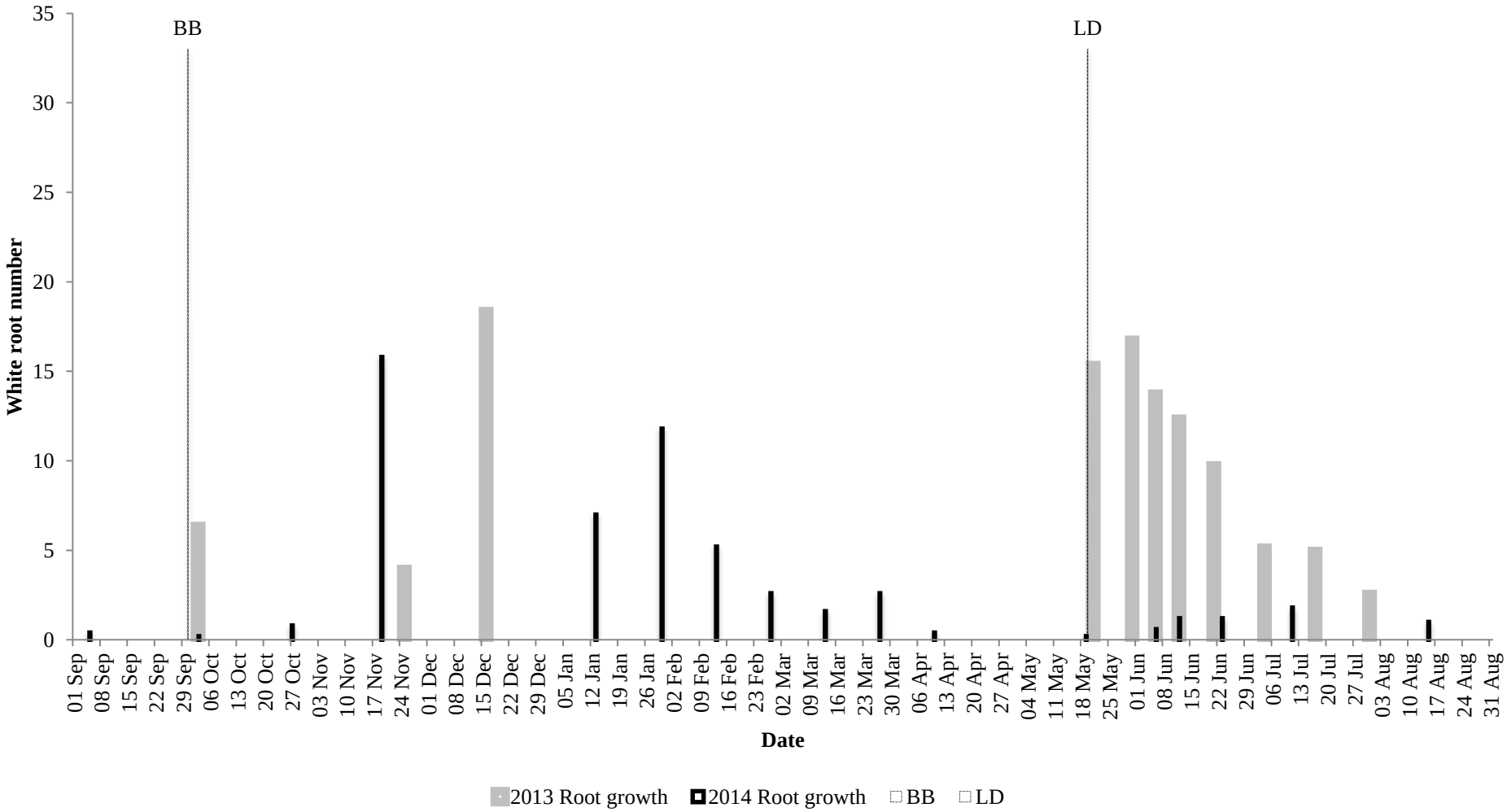

Fig 6. The seasonal change in average white root numbers in relation to phenological events, including bud break (BB) and 50 % leaf drop (LD) from May 2013 until November 2014 for young non-bearing 'Corder Gala'/M7.

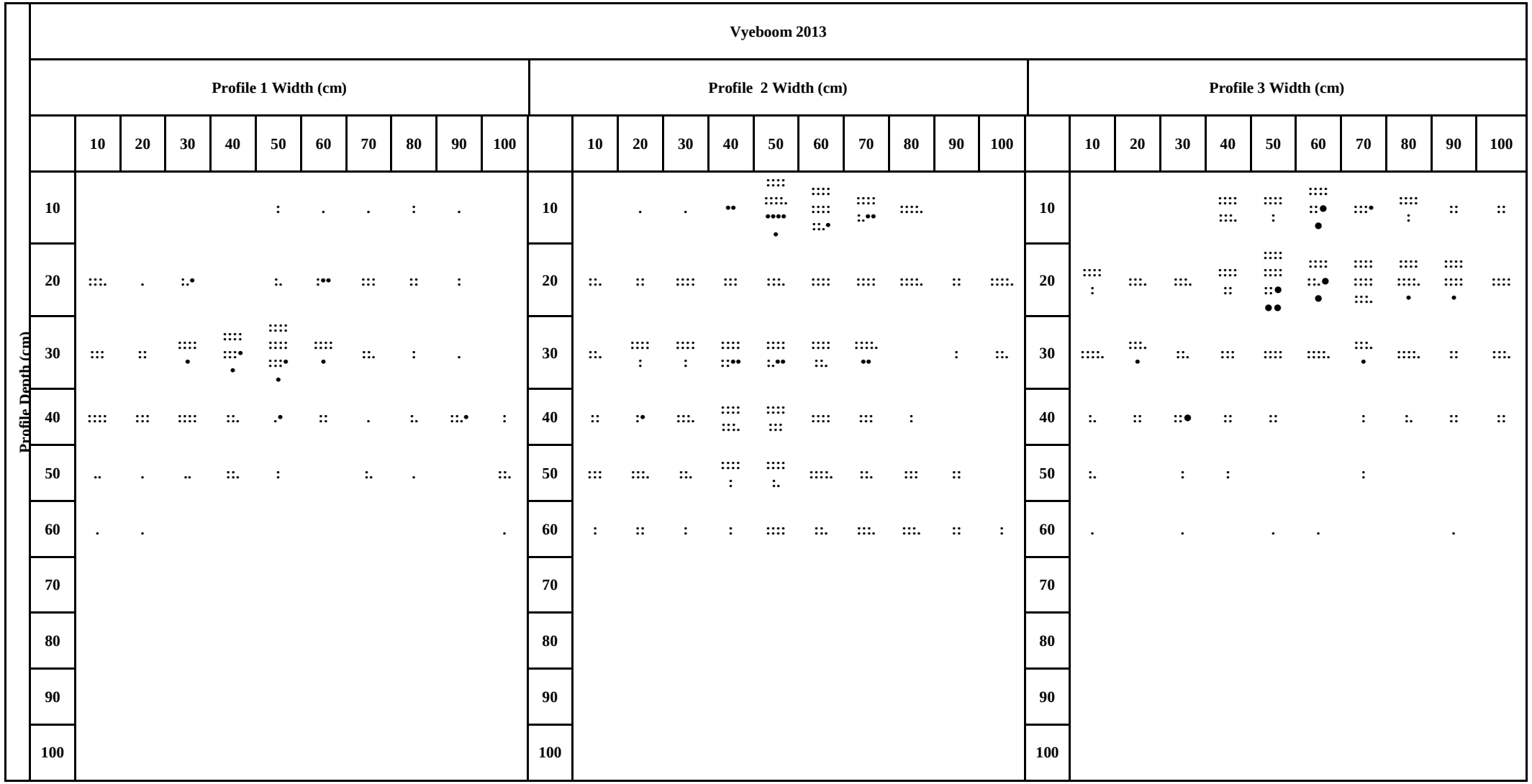

Fig 7. Fine root distribution of three 'Corder Gala' (M7) trees at 10 cm intervals down the 100 cm deep soil profile on 14 May 2013. Fine roots were characterized according root diameter** **::** = < 2 mm; **:** = 2-5 mm; • = 5-10 mm; ○ = > 10 mm

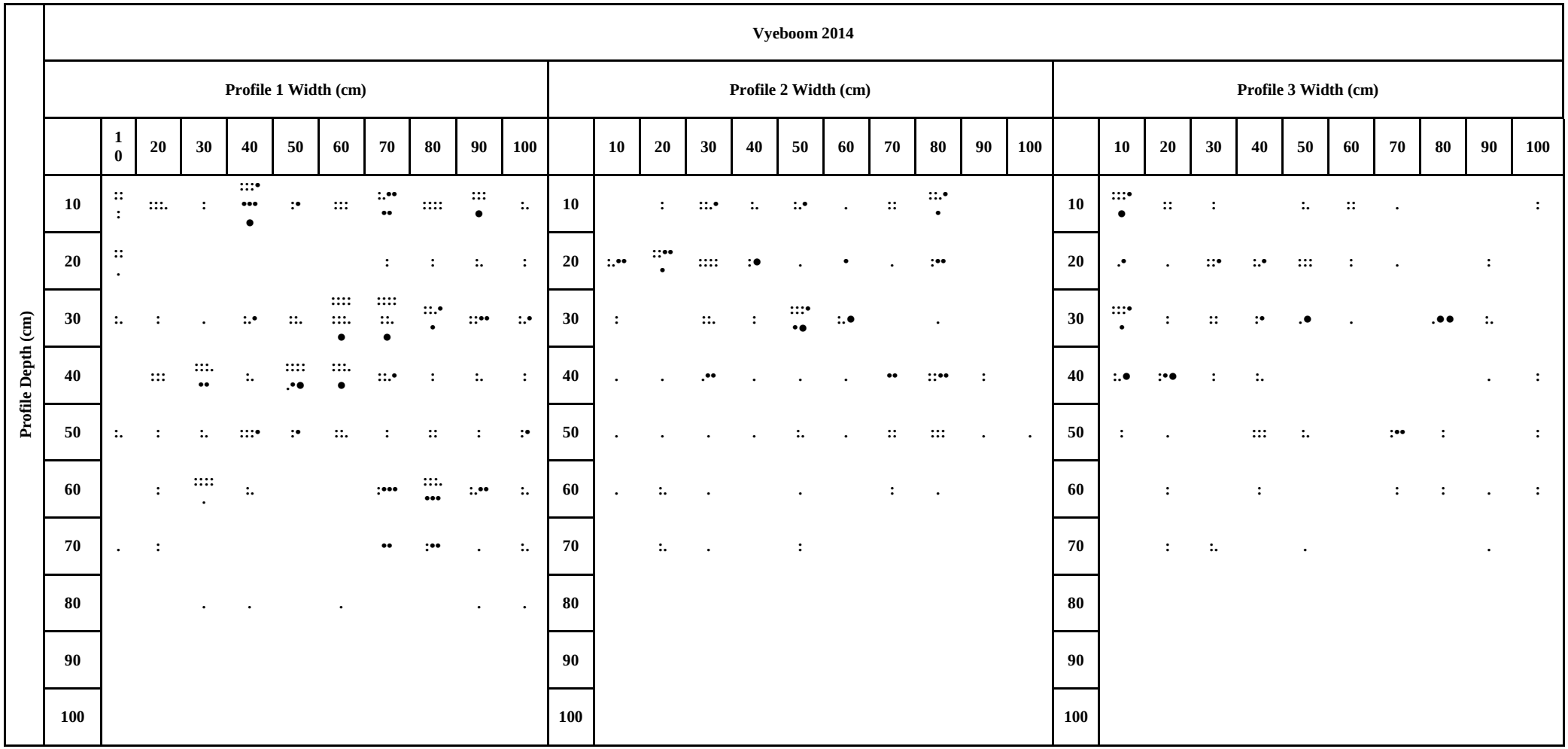

Fig 8. Fine root distribution of three 'Corder Gala' (M7) trees at 10 cm intervals down the 100 cm deep soil profile on 19 May 2014. Fine roots were characterized according root diameter* * **::** = < 2 mm; **:** = 2-5 mm; • = 5-10 mm; ○ = > 10 mm

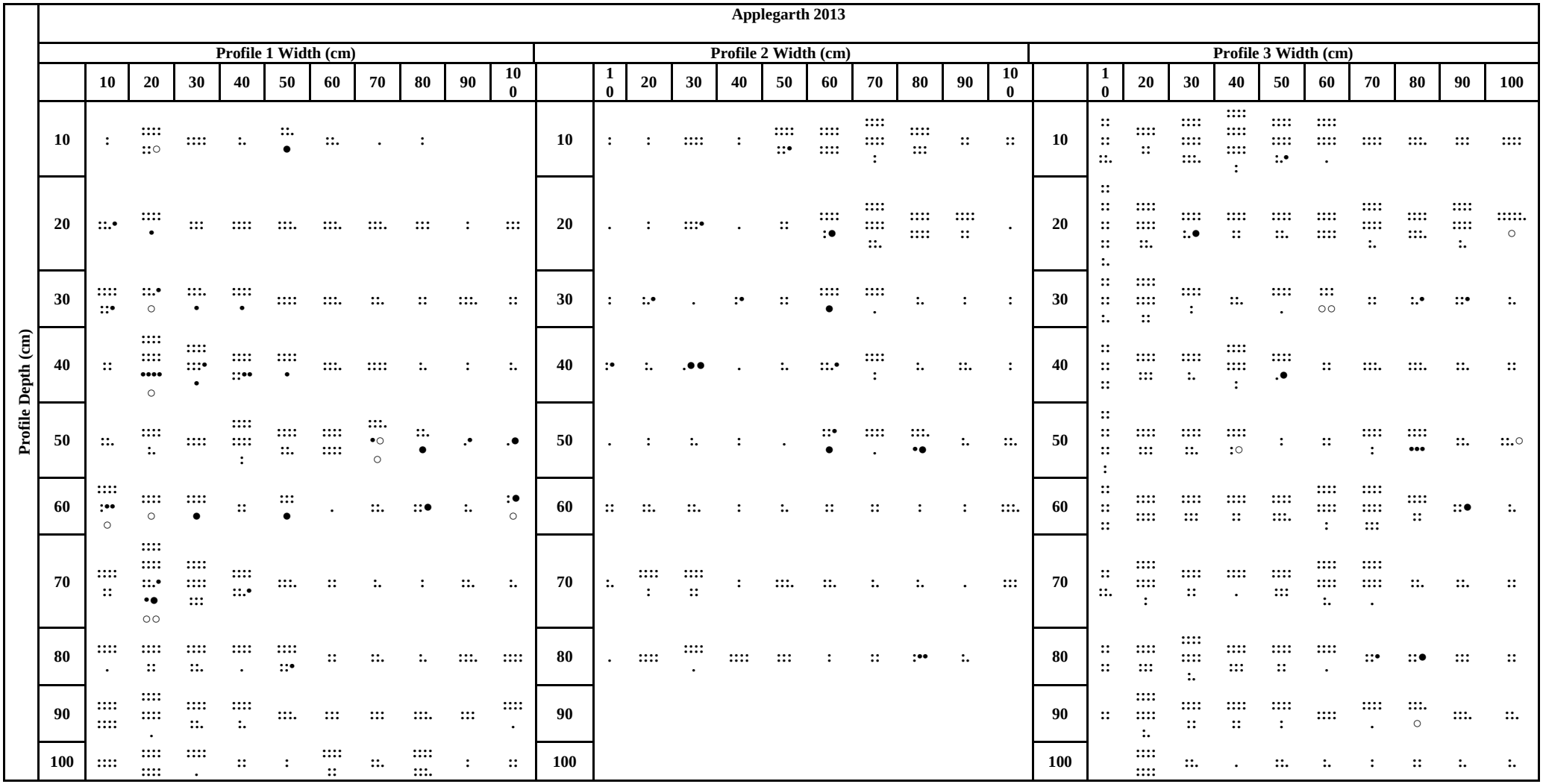

Fig 9. Fine root distribution of three 'Golden Delicious' (M793) trees at 10 cm intervals down the 100 cm deep soil profile on 5 June 2013. Fine roots were characterized according root diameter* * **::** = < 2 mm; **:** = 2-5 mm; • = 5-10 mm; ○ = > 10 mm

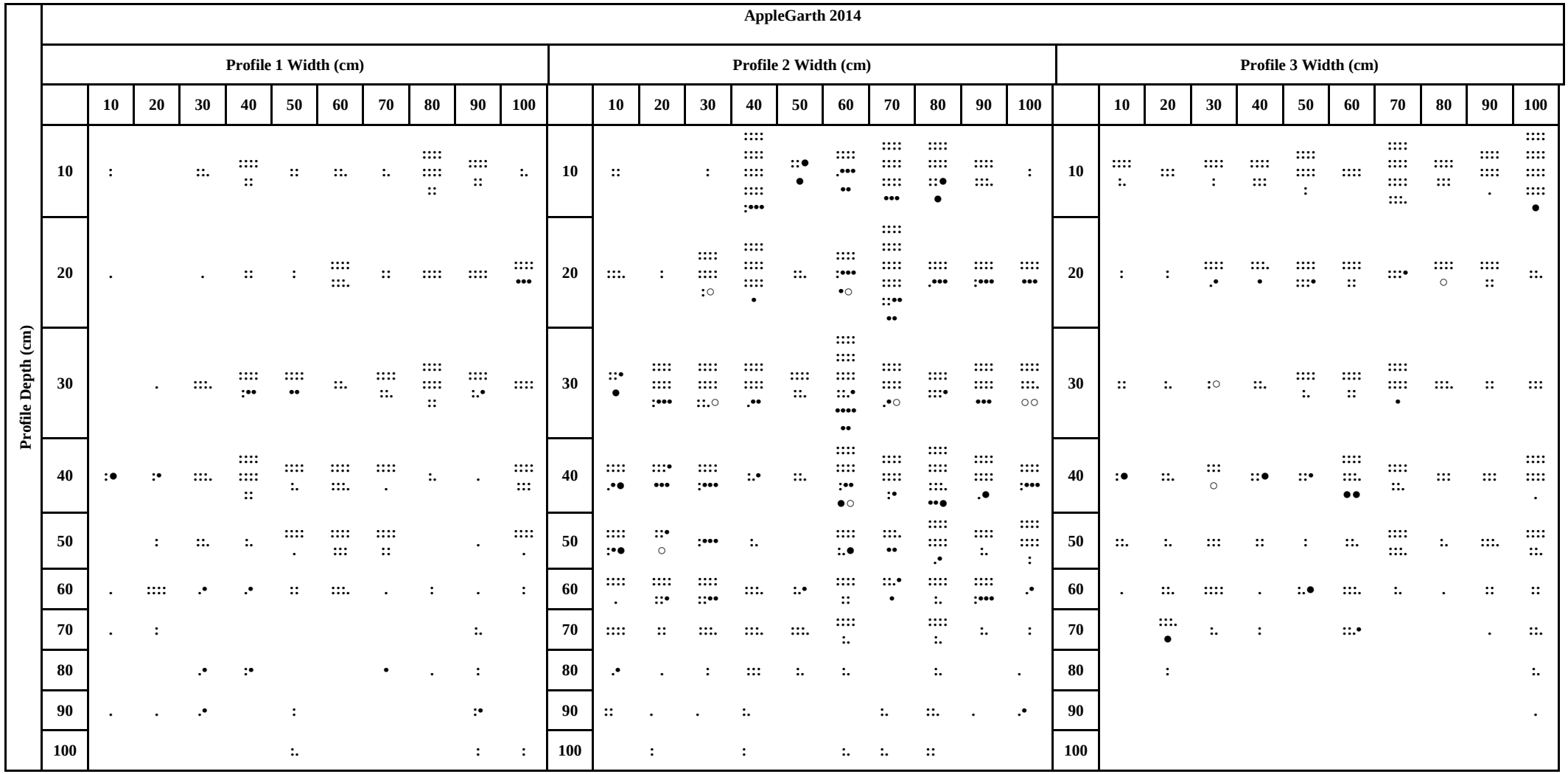

Fig 10. Fine root distribution of three 'Golden Delicious' (M793) trees at 10 cm intervals down the 100 cm deep soil profile on 12 May 2014. Fine roots were characterized according root diameter* * **::** = < 2 mm; **:** = 2-5 mm; • = 5-10 mm; ○ = > 10 mm

Paper 2

The relationship between apple root growth dynamics, tree physiology and environmental factors

Introduction

Factors influencing the dynamics of fine root growth in apple are important for improved fertilization scheduling, as well as for understanding assimilate partitioning in fruit orchards (Eissenstat et al., 2006). New fine roots of deciduous trees are initially white in color and produced mostly in periodic, relatively concentrated phases of activity throughout the season, especially for mature trees (Atkinson and Wilson, 1980; Kuhns et al., 1985). White roots play a very significant role in nutrient absorption, cytokinin synthesis, as well as in tree and ecosystem carbon dynamics (Baldi et al., 2010; Ma et al., 2013; Marschner, 1995; White, 2001). Fine root growth cycles can account for 20-70 % of net primary production in many tree species due to the high annual turnover rate (Rytter, 2013; Wells and Eissenstat, 2001; Withington, 2005).

The phenological pattern of shoot, leaf and reproductive growth is mainly determined by seasonal dormancy and its cyclic pattern is therefore relatively consistent. Root systems of woody perennials, however, do not become inherently dormant and growth patterns can therefore vary annually (Kozlowski et al., 1991; Psarras et al., 2000). The predominant limiting factor that restricts root growth during inactive phases is either environmental or physiological, depending on the interaction between the species and the particular environmental conditions (Kuhns et al., 1985; Vargas et al., 2015).

Factors that control the onset, duration and magnitude of a root growth cycle are complex and findings are often contradictory regarding the influence of soil water, soil temperature and physiological factors on root growth patterns (Côté et al., 1998; Montagnoli et al., 2014; Vargas et al., 2015). Soil water and temperature fluctuate in a relatively wide range before they become limiting and suppress or terminate a root growth phase entirely (Abrisqueta et al., 2008; Gregory, 2008; Joslin et al., 2001). The influence of soil water and -temperature on the actual initiation and eventual cessation of root growth is considered feasible under limiting environmental conditions and can be considered the main factor controlling root growth

patterns (Deans, 1979; Montagnoli et al., 2014; Tierney et al., 2003; Pregitzer et al., 2000). For instance, low soil temperatures (<4 °C) during winter, as well as mid-summer droughts, play a critical role in the timing of root growth flushes in deciduous trees (Kuhns et al., 1985). Soil water content alone can be the main driver of fine root dynamics in rainfall dependent crops of tropical regions (Chairungsee et al., 2013). However, under certain conditions where soil water and -temperature are not the main cause for the particular root growth pattern, the strong influence of soil water and -temperature on the morphology, physiology and lifespan of developing roots is still very evident (Kuhns et al., 1985; McCormack and Guo, 2014; McMichael and Burke, 1998; Nightingale, 1935). Increasing soil temperatures can significantly increase root elongation, respiration, maturation, tannin accumulation and mortality rates and this influence is more pronounced for roots of smaller diameter (Pregitzer et al., 2000). Generally, root growth rates increase with increasing temperatures under adequate soil water and nutrient conditions and decline when temperatures become supra-optimal (Bevington and Castle, 1985; Montagnoli et al., 2014). Soil water can also influence root development directly or indirectly by affecting soil temperature, soil aeration and nutrient availability (Psarras, 1999). Besides the influence of direct environmental soil factors such as water and temperature on root growth, endogenous tree physiological or phenological factors probably have the greatest influence on the timing and magnitude of a root growth cycle (Joslin et al., 2001). The annual pattern of endogenuously controlled root growth is therefore related to tree resource availability (Rogers and Head, 1969). This is especially in situations where the seasonal fluctuation of soil water and temperature are not limiting for root growth.

Root growth forms part of a highly integrated system of competing sinks, which all have a dynamic demand for photosynthates, either current or reserve forms of carbohydrates (Flore and Layne, 1999; Kozlowski, 1992). The partitioning of the latter depends on the synthesis, transport and accumulation of phytohormones, both shoot- and root-derived (Marschner, 1995; Rogers and Head, 1969). Root growth patterns are therefore strongly influenced by, and in many cases primarily determined by, sink interactions, carbohydrate availability and phytohormone signaling, which in turn are affected by environmental conditions (Maggs, 1963; Rogers and Head, 1969; Schupp and Ferree, 1990). A number of sinks compete for the available tree carbohydrates and tend to follow an allocation hierarchy, which is more pronounced in mature bearing trees, where above ground processes are favoured during the growing season (Flore and Layne, 1999). According to Flore and Layne (1999), dry matter

partitioning to roots decreases as trees begin to bear fruit and increase in age. Strong periodicity in root growth is therefore more evident in fruiting trees, as compared to young non-bearing trees, due to increased competition for assimilates (Palmer, 1992; Yao et al., 2006).

Integrated experiments are needed to shed light on the dynamics of fine root growth, which is inextricably linked to whole tree physiological processes and the prevailing soil environment. Isolated studies of roots or shoots cannot account for the very complex whole tree carbon allocation patterns of woody perennials (Pregitzer, 2003). The importance of timing of white root production in deciduous trees is further accentuated by the difficulty in quantifying root activity due to the inaccessibility and ephemeral nature of fine roots (Withington, 2005). Minirhizotrons (MR) have proved to be an excellent tool for monitoring root growth over time, as they allow multiple in situ observations at a particular site, in order to determine seasonal patterns (Eissenstat et al., 2006; Gluzek et al., 2013; Withington et al., 2003).

Although there is evidence that white root growth (carbon (C) acquisition) is regulated by either soil environmental conditions (Kuhns et al., 1985; Burke and Raynal, 1994), C availability (source regulation) (Joslin et al., 2001) or C demand by the roots (sink regulation) (Kaschuk et al., 2009), none of these theories are integrated and satisfactory (Farrar and Jones, 2000). The control over activity and growth of roots and above ground plant parts involves a variety of factors distributed throughout the plant and is referred to as ‘shared’ control (Farrar and Jones, 2000; Côté et al., 1998). C acquisition by roots for growth is therefore complex and highly unpredictable as the environment, species, tree age and tree history all play a role in the timing of root growth. Root growth patterns of apple orchards may be more complex than in natural deciduous forests, as additional variance can arise from the great variety of scion-rootstock combinations, as well as the crop (fruit), which is further subjected to different cultural practices (Ma et al., 2013; Rogers and Head, 1969). Furthermore, there are few reports on apple root growth dynamics in either the Southern hemisphere or in a Mediterranean climate which is generally characterized by different climatic conditions than what reports from the Northern hemisphere describe.

The objectives of this paper were 1) to investigate the possible influence of soil temperature and soil water fluctuations on white root numbers, 2) determine if a soil thermal limitation to apple root growth exists under these particular orchard conditions, 3) to describe the possible effects of the soil environment on the qualitative aspects of root growth and 4) to determine if

a relationship exists between white root growth and photosynthesis under field conditions. If white root production is directly influenced by/ or has a direct influence on photosynthetic rates, it would improve our understanding of resource management of white roots and may partly explain seasonal fluctuation in photosynthesis.

Materials and methods

Experimental sites

Site 1

The study on mature 'Golden Delicious' apple trees, grafted onto M793 rootstock, was carried out on the commercial farm 'Applegarth' (S 34° 08'10.2" E 019° 02'04.4") in the Elgin region of Western Cape, South Africa. The orchard was planted on a heavy clay loam soil with a 50 % stone fraction. The orchard was established in 2007, with trees planted 2 m apart with a row spacing of 4.5 m. The orchard was managed according to standard commercial cultural practices. Trees were irrigated with micro jets positioned between trees on the tree line. Irrigation scheduling was performed more or less on an *ad hoc* basis by the farm management using evapotranspiration data. Weed control consisted of mowing the grass between the rows during summer and using glyphosate once a year, usually after mowing. This weed control strategy was insufficient in controlling weed and grass growth within the orchard and weeds were present throughout most of the year. This trial was part of a bigger fertilization experiment with a randomized complete block design (Paper 3). Root growth, hourly soil water and temperature readings and photosynthesis measurements were performed on nine replicates only. Each replicate consisted of a single tree in a block of two trees, monitored by a single MR tube and soil water probe.

Site 2

The study on young 'Corder Gala' apple trees on M7 rootstock was performed on a newly established orchard on the commercial farm 'Vyeboom Plaas' (S 34° 05'19.8" E 019° 05'24.7") in the Vyeboom area of the Western Cape, South Africa. The trees that were used for root observations in this study formed part of a larger cover crop experiment carried out by

the research institute, ARC Infruitec-Nietvoorbij in the form of a randomized complete block design. However, the cover crops were only sown during the second season of the trial and no treatment effect was yet observed. The orchard was planted in the spring of 2012 on a sandy loam soil, with trees 2 m apart and a row spacing of 4 m. Trees were irrigated using micro jets with one micro jet positioned between two trees. Irrigation scheduling was based on neutron moisture probe readings performed by Nietvoorbij technicians in order to maintain soil water at field capacity. However, irrigation was seldomly performed due to the soil remaining too wet (determined by neutron moisture readings) throughout most of the first season. Each replicate in this study consisted of a single tree at which one MR tube and soil moisture probe was installed. Root observations, hourly soil water and temperature readings, as well as photosynthesis measurements, were obtained for five replicates.

Soil water and temperature

Continuous logging capacitance probes (DFM, Continuous logging Soil Moisture Probe, DFM Software Solutions CC, Penhill, South Africa and Aquacheck (Pty) Ltd Soil Moisture Probes, Durbanville, South Africa) measured soil water and temperature at 10 cm intervals from the surface to a depth of 60 cm on an hourly basis for both the young and mature apple sites. Each probe was installed within the distribution area of the micro jets, 50 cm from the trunk, and approximately 30 cm from the tree line towards the work row. The relative soil water % measured by the capacitance based probes was validated by neutron water readings in the young apple orchard and gravimetric soil water content measurements in the mature apple orchard. Neutron water measurements were performed by the ARC Infruitec-Nietvoorbij on a weekly basis at soil depths of 30, 60 and 90 cm. Soil samples for determining the gravimetric soil water contents were collected in the top 40 cm soil around three of the nine replicates (soil moisture probes), at depths of 10, 20-30 and 40 cm during January 2014. Water loss from the samples was prevented during transport from the orchard to the laboratory where the samples were weighed to obtain wet mass (*Mw*). Samples were then oven dried and weighed again to obtain dry mass (*Md*). Gravimetric water content (w) was then calculated with the following equation:

$$w\ (\%) = \frac{Mw - Md}{Md} \times 100$$

Root growth data collection

Data on white root numbers were obtained through the analysis of MR images taken with a root scanner (CI-600, CID Bioscience, Inc, Camas, WA USA). A single 1.05 m acrylic butyrate tube was installed at each replicate tree at a 45° angle, 40 cm from the trunk, parallel to the work row. Tube installation for the mature orchard took place on 15 April 2013, five weeks before the first root scan. Installation in the young orchard occurred on 4 April 2013, six weeks before the first root scan was taken. Root scans were taken throughout the year, on a weekly or bi-weekly basis, during the most active phases of root production and on a monthly basis during periods with less root growth. For example, if a MR observation revealed white root activity the observation frequency was increased. The MR observation frequency was similarly decreased as a root flush came to an end. The data collection process for a single tube consisted of scanning four "windows" or sections of the tube creating images representing more or less the following four different soil depths: 0-15 cm, 15 – 30 cm, 30 – 50 cm and 50 – 70 cm. The approximate depth of each observation window was calculated by using the length of the tube in the soil and its angle (approx. 45°) with respect to the soil surface.

Roots that appeared white on the images were counted (Paper 1, Fig. 1). White roots were considered functionally alive with the cortex intact and root browning was therefore used as the distinguishing criteria defining white root number peaks. Individual roots that were considered white were counted manually for each image. The general seasonal root growth pattern of each site was determined using the average white root numbers of the entire tube for all replicates.

Photosynthesis

Photosynthetic capacity was measured using a Li-Cor Li-6400 infra red gas analyzer (Licor Inc., Lincoln, NE). Photosynthesis was measured on a bi-weekly basis from late November 2013 until March 2014, concurrent with root observation dates. Two leaves per tree were measured where MR were installed.

In order to compare the photosynthetic capacities between leaves measured over time, the various parameters influencing photosynthesis were set at fixed values throughout all the

measurements. Carbon dioxide levels were set at 380 ppm, photosynthetically active radiation (PAR) at 1800 µmol m^{-2} s^{-1}, flow rate at 500 µmol s^{-1}, leaf temperature at 25°C and vapour pressure deficit (VPD) was maintained as close as possible to 1.5 kPa. Furthermore, enough time was allowed for the leaf to adjust to this particular suite of external conditions i.e. each measurement set was recorded when photosynthetic rate became stable and did not fluctuate more than 1 µmol $C0_2$ m^{-2} s^{-1}.

Results and discussion

Soil Temperature and root dynamics

Site 1: Mature, bearing trees

The annual soil temperature fluctuation at this site closely resembled changes in ambient temperature, especially for soil temperature at 10 cm depth (Appendix, Fig. 5). Maximum average soil temperatures of 26 °C were reached in the top 10 cm of soil during December to February (summer) (2013/14) (Fig.1). Minimum temperatures of 5-10 °C (at some probes <5 °C) were reached during June to August (winter) in the top 10 cm soil (Fig.1). Summer and winter soil temperatures for the 2014/15 season were similar to 2013/14 (data not shown). Daily minimum and maximum soil temperatures during summer (December-February) varied between 3 to 6 °C in the top 10 cm (Fig. 1). The deeper soil layers exhibited less fluctuation in temperature, resulting in higher minimum temperatures and lower maximum temperatures, which confirms previous findings (Faget et al., 2013). The maximum temperature of 22 °C at 60 cm soil depth was recorded during summer (February), whereas the minimum of 10 °C was recorded during winter months (June). Daily minimum and maximum soil temperatures also did not differ at 60 cm depth (Fig. 2), indicating that there is very little diurnal fluctuation in temperature at this depth. There was also a substantial seasonal delay in soil temperature changes with increasing depth. On average, the lowest white root numbers occurred in the upper 15 cm of the soil (Fig. 3 and 4). The considerable diurnal and day to day variation in soil temperature in the top soil layer could be responsible for the lower root numbers at this depth, as short-term soil temperature fluctuations have been reported to be detrimental to root growth and survival (Nielsen, 1974; Pregitzer et al., 2000).

The range of fluctuation in soil temperature depends on soil texture, pore space and organic matter content as well as vegetation type (Nielsen, 1974; Pregitzer et al., 2000). As result, at this site, the majority of new root production occurred at a depth between 15 cm and 70 cm, especially for the period May 2014 to December 2014 (Fig. 4). During the same period in the previous season, the majority of white roots occurred slightly deeper, between 30 cm and 70 cm (data not shown). The difference in white root numbers between the 0-15 cm soil layer and the 50-70 cm layer was greatest during the 2014 summer root growth flush (Fig. 4).

Besides the contribution of lower variation in temperature with increasing depth to the promotion of white root growth observed at this site, soil water content was generally higher lower down in the profile (Table 1), which also seemed to promote root development. During the trial period, vigorous grass and weed growth (Appendix A, Fig. 6 and 7) in the tree row was often observed during the growing season at this site. Competition for water and nutrients from weed and grass roots closer to the soil surface could have also reduced apple root growth in the upper 10 cm. Delver (1980) reports that the topsoil under grass strips are drier and lower in nutrients due its dense root system which competes strongly for water and nutrients consequently leading to less tree roots in the top soil compared to herbicide strips.

According to Lyr (1996), soil temperature is a very important factor determining the growth of fine roots as species specific temperature ranges exist for optimum growth, with some authors reporting a good correlation between soil temperature changes and root growth patterns (Bevington and Castle, 1985; Burke and Raynal, 1994; Kuhns et al., 1985), and others not (Côté et al., 1998; Joslin et al., 2001). White root growth patterns in our study could not be attributed to the seasonal soil temperature dynamics, as changes in soil temperature did not correlate to changes in white root numbers within the 0 – 70 cm soil profile (Fig. 3). Under the conditions in this study, soil temperatures rarely dropped below 5°C in the top 10 cm, whereas the minimum recorded for 60 cm depth was 12°C. In soil depths below 10 cm, temperatures in winter were mostly above the minimum temperature of 7 °C required for apple (Delicious apple - Stayman variety) root growth (Nightingale, 1935). In this site, high white root numbers (flushes) were furthermore noted under the lowest and highest soil temperature periods (Fig. 3), again indicating the lack of correlation between soil temperature and the initiation and end of white root flushes. The potential for white root growth is therefore controlled by factors other than soil temperature in this site.

White root development is affected to some degree under a suboptimal temperature regime (Eissenstat et al., 2000), which was evident at this site (Fig. 3 and 4). Compared to the summer root growth peak, the persistence of white roots was more prolonged during the winter peak, when soil temperatures were lower, fluctuated less and soil water content was higher due to winter rains. This could have resulted in greater root cortex longevity, thereby postponing root browning and maturation (Kuhns et al., 1985; Nightingale, 1935). In general, young tree roots (including apple) at low temperatures are white, whereas roots growing at higher temperatures have an increased rate of browning and senescence (Eissenstat et al., 2000; Kaspar and Bland, 1992; Rogers and Head, 1969). Nutrient uptake dynamics could therefore differ from other regions, as white roots do not occur during winter due to soils being too cold whereas the warmer winter soil conditions at this site promoted cortex longevity and lowered the rate of root browning. Fine root tips were found to be rarely completely suberized (brown) under low (4-15°C) soil temperature conditions in black walnut trees (Kuhns et al., 1985) which corresponds to reports by Nightingale (1935) who found that apple roots remain white for weeks at soil temperatures below 18°C. As winter soil temperatures in our trial were within this cooler range (10 – 12°C at 60 cm depth), this may have further contributed towards the extended growth of white roots observed during winter.

Fundamental differences in root development between summer and winter can occur, as temperature affects branching patterns, elongation rates, mean root diameter and root turnover (Faget et al., 2013; Gregory, 2008; Kaspar and Bland, 1992; McMichael and Burke, 1998). Differences in root branching between two seasonal root flushes can therefore affect root numbers. The summer root growth peak in this site was more pronounced, but shorter than the winter peak. Increased root branching in summer as a result of higher soil temperatures, as reported by Faget et al. (2013), could partly explain the higher white root numbers in summer, because we did not differentiate between root order. The demography of root orders will therefore be different between the summer and winter flush and higher order roots have smaller diameters, resulting in lower average root longevity due to a higher percentage of finer roots as they generally have shorter life spans (Wells and Eissenstat, 2001).

In addition to the environmental effects on root numbers and life span, internal tree factors could also have contributed to the sharp decline in white root numbers in summer, compared

to the steadier decline in winter. Strong endogenous carbohydrate competition with fruit during summer is known to reduce new root production (Maggs, 1963; Palmer, 1992; Yao et al., 2006). Carbohydrates are preferentially allocated to fruit as the sucrose concentration increases during the cell expansion period (Ho, 1992; Li et al., 2012). Cell expansion commences from approximately 42 days (Bergh, 1990) to two months (Miqueloto et al., 2014) after full bloom and corresponds with the time when white root activity at this site rapidly decreased (Fig. 5). Therefore, fruit: root competition for available carbohydrates/photosynthate, together with the warmer and drier soil environment in this site, may partly explain the shorter white root peak in summer compared to winter.

Site 2: Young, non-bearing trees

Maximum summer soil temperatures at site 2 at 10 cm soil depth reached 35 °C in February, while the minimum at this depth dropped to 6°C during winter (July) (Fig. 6). The daily minimum and maximum soil temperatures during summer (December-February) often varied by 10 to 15°C in the top 10 cm, which is considered unfavorable for root development (Nielsen, 1974; Pregitzer et al., 2000). During winter (June-August), daily temperature fluctuated less (± 6 °C) than in summer. At 60 cm soil depth, the difference between the daily minimum and maximum temperatures was negligible (Fig 7). The maximum soil temperature (25 °C) at 60 cm was reached during summer (February) and the minimum temperature (12°C) was recorded during winter (July). These observations are expected in a sandier soil type with less buffer capacity for temperature and soil water variation.

Differences in root growth dynamics were evident between the top 0-15 cm and lower 50-70 cm soil layers (Fig 8). This difference coincided with distinct soil temperature dynamics. Soil temperatures at 10 cm depth fluctuated drastically compared to the temperature at 60 cm (Fig 6 and 7). White root numbers were highest during summer (2013/14) at deeper soil depths (especially 50-70 cm), with very little root growth observed at shallower soil depths (0-15 cm) during this time (Fig 8). Whilst there was no direct relationship between root growth and soil temperature patterns over the season, daily fluctuations in temperature seemed to play a role in the distribution of white roots within the soil profile, confirming results in the mature, bearing orchard with less white roots in the top 15 cm due to huge fluctuation. The tendency for root growth to occur at lower soil depths was more pronounced in this site as the degree of summer

soil temperature fluctuation at shallow depths varied by 10 – 15 °C which is substantially greater compared to only 3 – 6 °C for the clay loam soil of site 1. This may partly be due to the enhanced effect of sandier soils on temperature variation (Nicholson, 2011; Nielsen, 1974). The effective herbicide weed control within the tree rows in addition to the sparse canopy development of the young trees at this site also allowed for maximum exposure of the soil to radiation, which would have led to increased evaporation compared to the mature trees (site 1) (Nielsen, 1974; Yao et al., 2009).

In comparison to the bearing orchard (site 1), the tendency for root production during winter was less pronounced. Although a root flush was observed in winter of the first season (2013) (Fig. 9), negligible root growth was observed the following winter and was therefore most likely a wounding response caused by the MR tube installation (Côté et al., 1998; Cripps, 1970). The lack of root growth during winter could however not be linked to soil temperature as the minimum temperatures were not lower than at site 1 where winter root growth timing was consistent for two consecutive seasons.

The timing of root production phases at this site was inconsistent between seasons, whereas soil temperature dynamics were similar between seasons. Although significant root growth between 30 and 50 cm depth coincided with increasing soil temperatures during December 2013, root growth was minimal during the same period the following year, even though soil temperatures at 40 cm were similar to the previous year (Fig. 10). Because soil temperatures were not limiting to root growth, endogenous or other environmental factors, or a combination thereof, probably had a greater influence on the dynamics of root growth at this site – together with the limitation of the position of the MR tubes to accurately capture this dynamic growth of young tree root development.

Soil water content and root dynamics

Site 1

Soil water content at site 1 differed amongst the nine plots monitored, partly due to the poor groundcover management and sub-optimal performance of the varying micro-jet delivery at

the different replicates (data not shown). Relating soil water content to white root growth was therefore performed individually, per replicate, and specific depth intervals.

Soil water content and white root growth dynamics differed between the two seasons when individual trees were compared. For example in one MR tube (tree), during the 2013/14 season, two prominent white root growth flushes at 50-70 cm depth was contrasted by minimal white root growth the following year (Fig. 11). This difference in white root growth coincided with differences in soil water content during the same period as a higher soil water content was observed during 2013/14. According to gravimetric soil water content analysis, the actual soil water % was substantially lower than determined by the soil water probes but followed the same trend with soil depth for all three replicates (Table 1). Kaspar and Bland (1992) and Joslin et al. (2001) reported similar observations of reduced white root growth as a resulting from a lack of water. However, variation in terms of white root growth dynamics was also evident for similar water regimes during 2013/14 in other observation trees, as well as between a high and low water content. For example, two trees (MR tubes) subjected to similar soil water contents produced different root growth patterns, where both a double (winter and summer) and single (summer only) peak in root growth occurred (Fig. 12 A and B). Under drier, but also constant soil water conditions, only a winter peak occurred during the same time period (Fig. 12 C). Soil water content was therefore not a good predictor of white root activity at this site and not the primary regulatory factor determining white root dynamics. This is in agreement with Abrisqueta et al. (2008) who found that, although reduced water availability decreased root growth, it did not alter the seasonal root growth dynamics in peach. However, when available soil water is the limiting factor for growth, i.e. either dry or saturated conditions, it can override all other factors that could potentially favor root growth (Abrisqueta et al., 2008; Kuhns et al., 1985; Psarras et al., 2000). The influence of soil water on the timing of root growth is therefore relative to other root growth controlling factors such as phenology (Gregory, 2008; Joslin et al., 2001) or yield.

At this site water did not seem to be a determining factor for the onset or end of white root flushes, therefore endogenuous tree factors appear to have the greatest influence in determining root growth patterns. Even though a higher soil water potential has been found to be strongly correlated to increased root elongation rates, phenological factors were able to override favorable soil water conditions and suppress elongation rates during certain times in apple and

other species (Eissenstat et al., 2006; Joslin et al., 2001; Pregitzer et al., 2000; Rogers and Head, 1969; Yao et al., 2006). Results at this site are in agreement with these studies.

Site 2

The fluctuation in soil water content between the five plots (probes) at site 2 were similar to each other (data not shown). Soil water content in general varied with soil depth. Average white root number was compared to soil water content at specific depths and times. When root growth of a single tree at a 30-50 cm depth was analyzed, white root numbers differed between two consecutive seasons (summer), even though changes in soil water showed similar trends (Fig. 13). However, low root numbers in the top 15 cm soil were associated with a lower soil water content and greater temperature fluctuations (discussed above) compared to the 50-70 cm soil interval (Fig 14). These factors most probably combined to impede root growth in the top 10 cm (Nielsen, 1974).

Similar to site 1, changes in soil water content was therefore not a good predictor of white root dynamics. This again suggests endogenous control of root growth e.g. available carbohydrates, which is able to override favorable soil water conditions as reported by Joslin et al. (2001).

Photosynthesis and root dynamics

Site 1

White root dynamics and periodic photosynthetic measurements (Appendix, Table 1) were compared between the nine individual replicate trees. Differences in photosynthetic rates did not correspond to changes in white root numbers (Fig 15). Photosynthesis decreased substantially from 23.1 to 13.6 (μmol CO_2 m^{-2} s^{-1}) during summer (late November 2013), while white root growth increased. After the sharp decline, photosynthesis increased again to 16.1 (μmol CO_2 m^{-2} s^{-1}) and subsequently fluctuated more conservatively until a slight increase before harvest (4 March 2014), followed by a decrease after harvest. Whilst, photosynthesis continued until late autumn, white root growth decreased steadily towards late summer (February 2014) and increased again after harvest. Photosynthesis was maintained by intact leaves at least until after 50 % leaf drop (data not shown) in late May. Although current

photosynthate production may support root growth during summer, other stronger sinks (shoot and fruit growth) may mask the direct influence of root growth on measured photosynthetic rates during this time (Flore and Layne, 1999) on mature, bearing trees. A direct correlation between root activity and photosynthesis is seldom evident, as photosynthate export from leaves is often independent of photosynthetic rates (Farrar and Jones, 2000). Furthermore the processes that control carbon acquisition and subsequent growth of roots are complex, as root dry matter accumulation, metabolic activity, reserve utilization and environment can also cause variability in photosynthate export to roots (Farrar and Jones, 2000).

Root activity patterns has been correlated with photosynthetic activity (Kozlowski, 1992; Zhou and Quebedeaux, 2003). Carbon allocation rates were not measured in this trial, thus it is not known if root growth during summer utilized current carbohydrates for growth or whether stored resources were used. However, the white root growth observed in winter in this study would have been dependent on reserve carbohydrates (Gaudinski et al., 2009). As trees age, root growth becomes more dependent on reserve carbohydrate sources and tree C allocation patterns become more complex as a result (Kozlowski, 1992) and therefore no evident correlation between Pn and white root growth could be established in this trial.

Site 2

In contrast to the mature, bearing trees, white root dynamics and average photosynthetic rates (Appendix, Table 2) followed similar trends in individual young trees at the non-bearing site. Both photosynthesis and white root numbers were low during late November, with the first corresponding measurement date, 25 November 2013 (Fig. 16). Both white root numbers and photosynthesis were substantially higher on 16 December 2013. Changes in photosynthesis and white root growth dynamics continued with a similar pattern for subsequent measurement dates until April 2014, indicating a possible relationship between photosynthesis and the onset, as well as duration, of white root growth. This is in agreement of reports of a correlation between root growth of maple tree seedlings and photosynthetic rates during their first year of growth (Kozlowski, 1992).

In contrast to bearing trees, young non-bearing trees have less complicated sink allocation demands and therefore less endogenous competition for photosynthates, due largely to the absence of reproductive structures (Flore and Layne, 1999; Kozlowski, 1992, Maggs, 1963).

Furthermore, root growth during the early stages of tree establishment have a stronger photosynthate demand, as a greater majority of the new primary growth become structural roots, as compared to mature trees were the majority of new roots are short-lived, fibrous feeder roots (Flore and Layne, 1999; Hudges and Gandar, 1993; Wells and Eissenstat, 2003). The potential for a measureable source (photosynthesis) response to root activity might therefore be greater in younger trees, due to higher energy demand from the larger number of "pioneer roots" produced. Young trees thus not only produce roots that demand more photosynthates than mature trees, they also produce relatively more roots which are less dependent on reserves (Hudges and Gandar, 1993; Polverigiani et al., 2011; Wells and Eissenstat, 2003). Thus, for young non-bearing apple trees under these conditions, active root growth seems to be a dominant sink for current photosynthates, hence the relationship between photosynthetic rates and root growth.

Root growth in relation to phenology

Site 1

White root growth was low during bud break (mid September), with activity increasing after full bloom (mid October), but a substantial increase only occurred after fruit set (beginning November) (Fig. 5). This trend was more prominent in 2014 than 2013. White root activity during the same time in 2013 was lower, but this may have been partly due to the temporary change of equipment (web camera vs. root scanner). Compared to data from the Root Scanner, the viewing area of the web camera lens is approximately 10 times smaller which also had to be directed manually within the MR tube potentially causing an underestimation of white root numbers in addition to the inappropriate angle of the lens, which reduced image quality. After fruit set in 2014, white root activity remained high during November and early December, but decreased substantially by 18 December 2014. A high number of white roots was therefore observed during shoot elongation, especially of long shoots, and the first phase of cell expansion in fruit. However, during the time when fruit sink activity was high in 2014, white root numbers remained low, decreasing to a minimum 3 weeks before harvest. Carbohydrate demand by fruit increases during the cell expansion phase of fruit growth (Ho, 1992; Li et al., 2012), which corresponds to the time when the summer peak of white roots rapidly declined (Fig. 5). According to Bergh (1990) and Miqueloto et al. (2014), fruit cell expansion commences between 42 days and 2 months after full bloom which requires substantial

resources. Furthermore the decrease in shoot extention growth occurred during the time when new root production was low (late December), which agrees with reports from Cripps (1970) who found that a tendency for root growth to decline with shoot growth in bearing apple trees. At harvest (beginning March 2014), white root activity was still very low, but approximately 2 weeks after harvest (mid March), root growth increased substantially. Unlike tree canopy growth, root growth of deciduous trees can occur throughout the year as the root system does not become inherently dormant (Kozlowski et al., 1991; Kuhns et al., 1985). This was reflected in the number of white roots which were fairly constant during April, substantially increasing around 50 % leaf drop (May) and reaching a maximum early in June 2014. The post-harvest root growth flush of 2013 was higher in magnitude and peaked later in June 2013 than in 2014, but trends were similar for the two seasons. White root growth was therefore substantial during tree dormancy, which is unexpected and in contrast with reports from the northern hemisphere (Atkinson and Wilson, 1980; Eissenstat et al., 2006; Psarras et al., 2000; Rogers and Head, 1969; Yao et al., 2006). In most cases white root survival during winter is very low (Wells and Eissenstat, 2001). However, very early reports indicate that under warm climates, root growth can occur during winter (Harris, 1926, 1929 in Cripps, 1970).

White roots in this site only decreased to low numbers (Fig. 5) by mid August and remained low during early spring (September – October). Soil temperature could not have been the primary cause for the low spring white root activity, as early spring conditions resembled that of late autumn (Fig. 1 and 2), when white root growth occurred consistently in this orchard (Fig. 5). The lack of white root growth during early spring could be attributed to two factors, both relating to endogenous tree conditions. Firstly, the high internal sink competition between actively growing tissues (Côté et al., 1998), coupled with the late winter root growth peak in this orchard, could have limited carbohydrate reserves for white root production early in the season. Furthermore, although synchronous growth for roots and shoots have been reported for both bearing and non-bearing trees (Cripps, 1970), asynchronous growth seems more common (Abrisqueta et al., 2008; Atkinson and Wilson, 1980; Bevington and Castle, 1985; Fumey et al., 2011; Ma et al., 2013). The continued reduction in root growth leading up to harvest, with negligible activity during high fruit growth, further indicates endogenous control of root growth (Côté et al., 1998; Yao et al., 2006). A reduction in root growth due to limited available photosynthates, resulting from competition with fruit, has previously been reported (Abrisqueta et al., 2008; Flore and Layne, 1999; Kaspar and Bland, 1992; Palmer, 1992; Yao et al., 2006) and thus confirms our results for site 1, in which the current fruit crop seems to be

the main internal factor suppressing fine root production between January and March (summer) (Fig. 5).

Crop load is known to consistently suppress root growth in apple, as fruits are considered priority sinks (Cannell, 1985; Palmer, 1992). At site 1, the post-harvest root growth flush, initiated 2-3 weeks after harvest, for two consecutive seasons (2013, 2014), and most likely occurred because of the elimination of competition through fruit removal. Most authors indicate the absence or lowest numbers of root activity during the month before harvest (Atkinson and Wilson, 1980; Eissenstat et al., 2006; Rom, 1996), with the exception of Psarras et al. (2000) who found that the major phases of fruit, shoot and root growth partially coincided.

Although the yield of this orchard was relatively low (average 50 $t.ha^{-1}$), the effect on crop load was quite evident when severe pruning in which mainly thinning cuts were applied followed the change of the training system from a central leader to solax during the winter of 2014 (52 $t.ha^{-1}$), resulting in a much reduced yield during 2015 (40 $t.ha^{-1}$). The reduced canopy size caused by pruning (2014) may have resulted in the slightly smaller post-harvest root growth peak of 2014. It is likely that less carbohydrates were allocated to the root system due to the imbalance and consequent restoration of the root : shoot ratio following pruning (Fumey et al., 2011; Gregory, 2008).

Carbohydrate reserves in the permanent parts of the tree (especially the root system) are at maximum around leaf drop (Kozlowski, 1992). However, post-harvest root growth might have been partially supplied by current photosynthesis, as opposed to entirely reliant on reserve carbohydrates for energy during this time. Leaf drop in this orchard did not occur abruptly, as in the Northern hemisphere, but over an extended period of time (50% leaf fall achieved 2-3 months post-harvest), during which a substantial amount of leaves were still photosynthesizing, thereby possibly allowing further white root growth. The effect of extensive white root growth during dormancy should receive more attention under local conditions in future as it has implications for stored carbohydrate reserves. Suboptimal carbohydrate reserves early in the season may affect overall orchard productivity as early reproductive growth in apple utilizes reserve carbohydrates (Loescher et al., 1990). However, new growth in young apple trees were found to be more dependent on N reserves than carbohydrates (Cheng and Fuchigami, 2002). White root growth during winter may therefore possibly affect tree N status positively through

increased uptake and together with cytokinin synthesis by white roots (Ma et al., 2013), root production during dormancy might be beneficial to reproductive development.

Site 2

Bud break was noted in late September to early October and at this time, low to negligible white root numbers were observed in both 2013 and 2014. White root growth dynamics varied notably from 2013 to 2014 and were inconsistent between the two consecutive seasons (Fig. 9). White root growth decreased from its summer peak in 2014 (end January), was absent before the start of leaf drop and remained low during winter 2014. A winter growth flush was observed the previous year, although it occurred fairly soon after MR tube installation and was possibly a wounding response, also reported by Côté et al. (1998). In contrast to site 1, white root activity was not noticed during the post-harvest root distribution studies of 2013 and 2014. Root growth, as determined by MR images were not representative of the actual number of white roots at this site as root excavations revealed root activity beyond the tube area, not observed in the MR. This was especially true during the 2014/15 season. As discussed in Paper 1, this was mainly attributed to the low root length density of apple trees younger than four years in addition to the greater amount of pioneer roots – which grow further away from the tree base than shorter feeder roots – produced by young trees compared to mature trees (Hudges and Gandar, 1993; Polverigiani et al., 2011). This leads to a low root interception by the MR tube window as observed by Yao et al. (2006). Additional MR tubes placed at various distances from the tree base should therefore improve root observation studies in young non-bearing apple trees.

Conclusion

A bimodal root growth trend was established for mature bearing ‘Golden Delicious’ apple trees. The first peak in root production occurred during summer (prior to the major fruit growth stage but overlapping with shoot growth) and a second flush in autumn/winter following harvest, which peaked during tree dormancy. Although a root flush in summer and early autumn (following fruit harvest) has been observed in apple (Cripps, 1970; Eissenstat et al., 2006; Rom, 1996; Yao et al., 2006), a winter flush during tree dormancy has not been previously reported, except for newly planted trees and early reports from Harris (1926)(in Cripps, 1970) under warm climates.

In the non-bearing 'Corder Gala' site, no consistent trend was observed over two seasons, although a consistent potential for root growth throughout the growing season was observed under these conditions which confirms reports from Cripps (1970) who observed continuous root growth from mid-spring until late autumn. In this trial, changes in root growth dynamics of mature, bearing trees and young, non-bearing trees on M793 on two different soil types, could not be attributed solely to soil environmental factors (temperature and water content), but favoured the hypothesis of endogenous control through carbohydrate availability and/or hormonal regulation. The root growth flush during winter, as well as the poor correlation between root growth and soil temperature dynamics, contradicts present perceptions that apple white root growth is dependant on soil temperatures and should become inactive during winter. The persistant winter flush can be attributed to the relatively warmer soils of the Western Cape region in winter compared to other apple producing regions in the Northern Hemisphere, as well as a possible additional contribution of active leaves until late in autumn. The extended root activity during winter may therefore affect tree dormancy due to hormone production by roots, as well as carbohydrate reserve availability in spring. Quantifying the effects of hormone (cytokinin) production by white root tips, nutrient uptake and reserve consumption by new roots during dormancy could reveal potential implications of the substantial winter root production in the Elgin region for orchard productivity.

New root production increased with soil depth, with minimal root growth in the top 15 cm soil. This could be attributed to the high, short term temperature fluctuations, lower soil water content and probably competition with weeds in the top 10 cm of the soil. The optimum efficacy of surface applied fertilizers to utilize the top roots under these conditions are therefore questioned at these sites, especially for the clay soil. Root growth should be promoted by implementing suitable ground cover management practices aimed at stabilizing soil temperature and water conditions, to increase white root numbers in the upper soil layer for improved nutrient use efficiency and long term tree performance (Atkinson and Wilson, 1980; Yao et al, 2009). Evidence from the non-bearing site also suggests a relationship between white root growth and photosynthesis, although it was not observed in the mature site, where it was probably masked by the more complex endogenous carbon allocation patterns.

References

Abrisqueta, J. M., Mounzer, O., Alvarez, S., Conejero, W., García-Orellana, Y., Tapia, L. M. and Ruiz-Sánchez, M. C. 2008. Root dynamics of peach trees submitted to partial rootzone drying and continuous deficit irrigation. *Agricultural water management 95*(8), 959-967.

Alvarez-Uria, P. and Körner, C. 2007. Low temperature limits of root growth in deciduous and evergreen temperate tree species. *Functional Ecology 21*(2), 211-218.

Atkinson, D. and Wilson, S.A. 1980. The growth and distribution of fruit tree roots: some consequences for nutrient uptake. (eds.) Atkinson, D., Jackson, J. E., Sharples, R. O. and Waller, W. M. *Mineral nutrition of fruit trees*, Butterworths publishers, 1980, p. 137-150.

Baldi, E., Wells, C. E. and Marangoni, B. 2010. Nitrogen absorption and respiration in white and brown peach roots. *Journal of Plant Nutrition* 33, 461-469.

Bergh, O. 1990. Effect of time of hand-thinning on apple fruit size. *South African Journal of Plant and Soil 7*(1), 1-10.

Bevington, K. B. and Castle, W. S. 1985. Annual root growth pattern of young citrus trees in relation to shoot growth, soil temperature, and soil water content. *Journal of the American Society for Horticultural Science 110*(6), 840-845.

Burke, M. K. and Raynal, D. J. 1994. Fine root growth phenology, production, and turnover in a northern hardwood forest ecosystem. *Plant and Soil 162*(1), 135-146.

Cannell, M.G.R. 1985. Dry matter partitioning in tree crops. In: Cannell, M.G.R.; Jackson, J.E., (eds.) Attributes of trees as crop plants. Abbotts Ripton, Institute of Terrestrial Ecology pp 160-193.

Chairungsee, N., Gay, F., Thaler, P., Kasemsap, P., Thanisawanyangkura, S., Chantuma, A. and Jourdan, C. 2013. Impact of tapping and soil water status on fine root dynamics in a rubber tree plantation in Thailand. *Frontiers in Plant Science 4*. p. 1-11.

Cheng, L. and Fuchigami, L. H. 2002. Growth of young apple trees in relation to reserve nitrogen and carbohydrates. *Tree Physiology 22*(18), 1297-1303.

Comas, L. H., Anderson, L. J., Dunst, R. M., Lakso, A. N. and Eissenstat, D. M. 2005. Canopy and environmental control of root dynamics in a long-term study of Concord grape. *New Phytologist 167*(3), 829-840.

Côté, B., Hendershot, W. H., Fyles, J. W., Roy, A. G., Bradley, R., Biron, P. M. and Courchesne, F. 1998. The phenology of fine root growth in a maple-dominated ecosystem: relationships with some soil properties. *Plant and Soil 201*(1), 59-69.

Cripps, J. E. L. 1970. A seasonal pattern of apple root growth in Australia. *Journal of Horticultural Science* 45, 153-161.

Deans, J. D. 1979. Fluctuations of the soil environment and fine root growth in a young Sitka spruce plantation. *Plant and Soil 52*(2), 195-208.

Delver, P. 1980. Uptake of nutrients by trees grown in herbicide strips. In: Mineral nutrition of fruit trees. Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M. Studies in the Agricultural and Food sciences. Butterworths, London – Boston. p. 229- 240.

Eissenstat, D. M., Wells, C. E. and Wang, L. 2000. Root efficiency and mineral nutrition in apple. In: *IV International Symposium on Mineral Nutrition of Deciduous Fruit Crops 564*, pp. 165-183.

Eissenstat, D. M., Lakso, A. N. Neilsen, D., Neilsen, G. H. and Smart, D. R. 2006. Seasonal patterns of root growth in relation to shoot phenology in Grape and Apple. *Acta Horticulturae 721*, 21 -26.

Faget, M., Blossfeld, S., Jahnke, S., Huber, G., Schurr, U. and Nagel, K. A. 2013. In: Plant roots: The hidden half, (eds). Eshel, A. and Beeckman, T, 4th edition, Taylor and Francis Group, CRC press, 31(1-8).

Farrar, J. F. and Jones, D. L. 2000. The control of carbon acquisition by roots. *New Phytologist 147*(1), 43-53.

Flore, J. A. and Layne, D. R. 1999. Photoassimilate production and distribution in cherry. *HortScience 34*(6), 1015-1019.

Fumey, D., Lauri, P., Guedon, Y., Godin, C. and Costes, E. 2011. How young trees cope with removal of whole parts of shoots: An analysis of local and distant responses to pruning in 1-year-old apple (*Malus* × *Domestica*; Rosaceae) trees. American Journal of Botany 98 (11), 1737-1751.

Gaudinski, J. B., Torn, M. S., Riley, W. J., Swanston, C., Trumbore, S. E., Joslin, J. D. and Hanson, P. J. 2009. Use of stored carbon reserves in growth of temperate tree roots and leaf buds: analyses using radiocarbon measurements and modeling. *Global Change Biology 15*(4), 992-1014.

Gluszek, S., Paszt, L. S., Sumorok, B., Derkowska, E. and Kozera, R. 2013. Application of the minirhizotron technique to studying the roots of fruit plants. *Advances in Science and Technology Research Journal* 7(18), 45–53.

Gregory, P. J. 2008. Plant roots: Growth, activity and interaction with soils. Blackwell Publishing Ltd. 65,131-137.

Ho, L. C. 2012. Fruit growth and sink strength. In: Fruit and seed production, Aspects of development, environmental physiology and ecology. (Ed) Marshall, C. and Grace, J. p. 101-117.

Hughes, K. A. and Gandar, P. W. 1993. Length densities, occupancies and weights of apple root systems. *Plant and Soil 148*(2), 211-221.

Joslin, J. D., Wolfe, M. H. and Hanson, P. J. 2001. Factors controlling the timing of root elongation intensity in a mature upland oak stand. *Plant and Soil 228*(2), 201-212.

Kaschuk, G., Kuyper, T. W., Leffelaar, P. A., Hungria, M. and Giller, K. E. 2009. Are the rates of photosynthesis stimulated by the carbon sink strength of rhizobial and arbuscular mycorrhizal symbioses? *Soil Biology and Biochemistry* 41, 1233–1244.

Kaspar, T. C., and Bland, W. L. 1992. Soil temperature and root growth. *Soil Science*, *154*(4), 290-299.

Kozlowski, T. T. 1992. Carbohydrate sources and sinks in woody plants. *The Botanical Review 58*(2), 107-222.

Kozlowski, T. T., Kramer, P. J. and Pallardy, S. G. 1991. The physiological ecology of woody plants, Academic press, inc. p.180-182.

Kuhns, M. R., Garrett, H. E., Teskey, R. O., and Hinckley, T. M. 1985. Root growth of black walnut trees related to soil temperature, soil water potential, and leaf water potential. *Forest Science 31*(3), 617-629.

Li, M., Feng, F. and Cheng, L. 2012. Expression patterns of genes involved in sugar metabolism and accumulation during apple fruit development. *PLoS One 7*(3), 33-55.

Loescher, W. H., McCamant, T. and Keller, J. D. 1990. Carbohydrate reserves, translocation, and storage in woody plant roots. *HortScience 25*(3), 274-281.

Lyr, H. 1996. Effect of the root temperature on growth parameters of various European tree species. In: *Annales des sciences forestières* 53(2-3), 317-323. EDP Sciences.

Ma, L., Hou, C. W., Zhang, X. Z. Li, H. L., Han, De G., Wang, Y. and Han, Z. H. 2013. Seasonal growth and spatial distribution of Apple tree roots on different rootstocks or interstems. *Journal of American Society of Horticultural Science* 138(2), 79–87.

Maggs, D. H. 1963. The reduction in growth of apple trees brought about by fruiting. *Journal of Horticultural Science* 38(2), 119-128.

Marschner, H. 1995. Mineral nutrition of higher plants second edition. London. Academic press, 152-157, 508-513.

McCormack, M. L. and Guo, D. 2014. Impacts of environmental factors on fine root lifespan. *Frontiers in plant science 5*, p. 1-11.

McMicael, B. L. and Burke, J. J. 1998. Soil temperature and root growth. *Hortscience* 33(6), p. 947-951.

Miqueloto, A., do Amarante, C. V. T., Steffens, C. A., dos Santos, A. and Mitcham, E. 2014. Relationship between xylem functionality, calcium content and the incidence of bitter pit in apple fruit. *Scientia Horticulturae 165*, 319-323.

Montagnoli, A., Di Iorio, A., Terzaghi, M., Trupiano, D., Scippa, G. S. and Chiatante, D. 2014. Influence of soil temperature and water content on fine-root seasonal growth of European beech natural forest in Southern Alps, Italy. *European Journal of Forest Research 133*(5), p. 957-968.

Nicholson, A. F. 2011. An investigation into factors in the root environment that affect growth and development of roots and the influence of ground covers on these factors. Masters Thesis in Horticultural Science, Stellenbosch University, South Africa.

Nielsen, K. F. 1974. Roots and root temperatures. In: The plant root and its environment. Edited by E. W. Carson, The University Press of Virginia. p. 293-322.

Nightingale, G. T. 1935. Effects of temperature on growth, anatomy, and metabolism of apple and peach roots. *Botanical Gazette* 96(4), 581-639.

Palmer, J. W. 1992. Effects of varying crop load on photosynthesis, dry matter production and partitioning of Crispin/M.27 apple trees. *Tree Physiology* 11, p. 19 – 33.

Polverigiani, S., McCormack, M. L., Mueller, C. W. and Eissenstat, D. M. 2011. Growth and physiology of olive pioneer and fibrous roots exposed to soil moisture deficits. *Tree Physiology 31*(11), 1228-1237.

Pregitzer, K. S. 2003. Woody plants, carbon allocation and fine roots. *New Phytologis 158*(3), 421-424.

Pregitzer, K. S., King, J. S., Burton, A. J. and Brown, S. E. 2000. Responses of tree fine roots to temperature. *New Phytologist 147*(1), 105-115.

Psarras, G. 1999. Effect of water and nutrient stresses on morphological and physiological characteristics of apple roots. Ph.D thesis presented to the Faculty of Graduate School of Cornell University.

Psarras, G., Merwin, I. A., Lakso, A. N. and Ray, J. A. 2000. Root growth phenology, root longevity, and rhizosphere respiration of field grown 'Mutsu' apple trees on 'Malling 9' rootstock. *Journal of the American Society for Horticultural Science* 125(5), 596-602.

Rogers, W.S. 1939. Root studies VIII. Apple root growth in relation to rootstock, soil, seasonal and climatic factors. *Journal of Horticultural Science* 17, 99–130.

Rogers, W.S. and Head, G.C. 1969. Factors affecting the distribution and growth of roots of perennial woody species, p. 111–148. In: W.J. Whittington (ed.). Root growth. Butterworths, United Kingdom

Rom, C.R. 1996. Coordination of root and shoot growth: roots and rootstocks. In: K.M. Maib, P.K. Andrews, G.A. Lang and K. Mullinix (eds.), Tree Fruit Physiology: Growth and Development. Good Fruit Grower, Yakima, Washington, USA, 53-67.

Rytter, R. M. 2013. The effect of limited availability of N or water on C allocation to fine roots and annual fine root turnover in Alnus incana and Salix viminalis. *Tree Physiology 33*, p. 924-939.

Schupp, J. R. and Ferree, D. C. 1990. Influence of time of root pruning on growth, mineral nutrition, net photosynthesis and transpiration of young apple trees. *Scientia Horticulturae 42*(4), 299-306.

Tierney, G. L., Fahey, T. J., Groffman, P. M., Hardy, J. P., Fitzhugh, R. D., Driscoll, C. T. and Yavitt, J. B. 2003. Environmental control of fine root dynamics in a northern hardwood forest. *Global Change Biology 9*(5), 670-679.

Vargas, O. L. 2015. Nitrogen Fertigation Practices to Optimize Growth and Yield of Northern Highbush Blueberry (*Vaccinium corymbosum* L.). A Ph.D. dissertation submitted to Oregon State University.

Wells, C. E. and Eissenstat, D. M. 2001. Marked differences in survivorship among apple roots of different diameters. *Ecology 82*(3), 882-892.

Wells, C. E., & Eissenstat, D. M. 2003. Beyond the roots of young seedlings: The influence of age and order on fine root physiology. *Journal of Plant Growth Regulators 21*, 324-334.

Withington, J.M., Elkin, A.D., Bułaj, B., Olesiński, J., Tracy, K.N., Bouma, T.J., Oleksyn, J., Anderson, L.J., Modrzyński, J., Reich, P.B. and Eissenstat, D.M. 2003. The impact of material used for minirhizotron tubes for root research. *New Phytologist 160*(3), 533-544.

Withington, J. M. 2005. Ph. D. Thesis in Ecology. Fine root production and lifespan in eleven temperate tree species growing in a common garden in Poland. Pennsylvania State University.

White, P. J. 2001. The pathways of calcium movement to the xylem. *Journal of Experimental Botany* 52(358), 891-899.

Yao, S., Merwin, I. A. and Brown, M. G. 2006. Root dynamics of apple rootstocks in a replanted orchard. *HortScience 41*(5), 1149-1155.

Yao, S., Merwin, I. A. and Brown, M. G. 2009. Apple root growth, turnover, and distribution under different orchard groundcover management systems. *HortScience 44*(1), 168-175.

Yocum, W. W. 1937. Root development of young Delicious apple trees as affected by soils and by cultural treatments. *Historical Materials from University of Nebraska-Lincoln Extension* p. 910.

Zhou, R. and Quebedeaux, B. 2003. Changes in photosynthesis and carbohydrate metabolism in mature apple leaves in response to whole plant source-sink manipulation. *Journal of the American Society for Horticultural Science 128*(1), 113-119.

Table 1: Soil water content (%) (according to capacitance based probes) compared to gravimetric soil water content for three replicates (plots) at three soil depth intervals.

Plot	Soil depth	Soil water content (%) (Probe)	Soil water content (%) (Gravimetric)
1	10	57.87	25.43
	20-30	58.45	29.18
	40	60.97	42.33
2	10	43.41	17.19
	20-30	36.64	23.50
	40	60.93	35.04
3	10	29.17	10.69
	20-30	55.15	28.13
	40	56.78	24.33

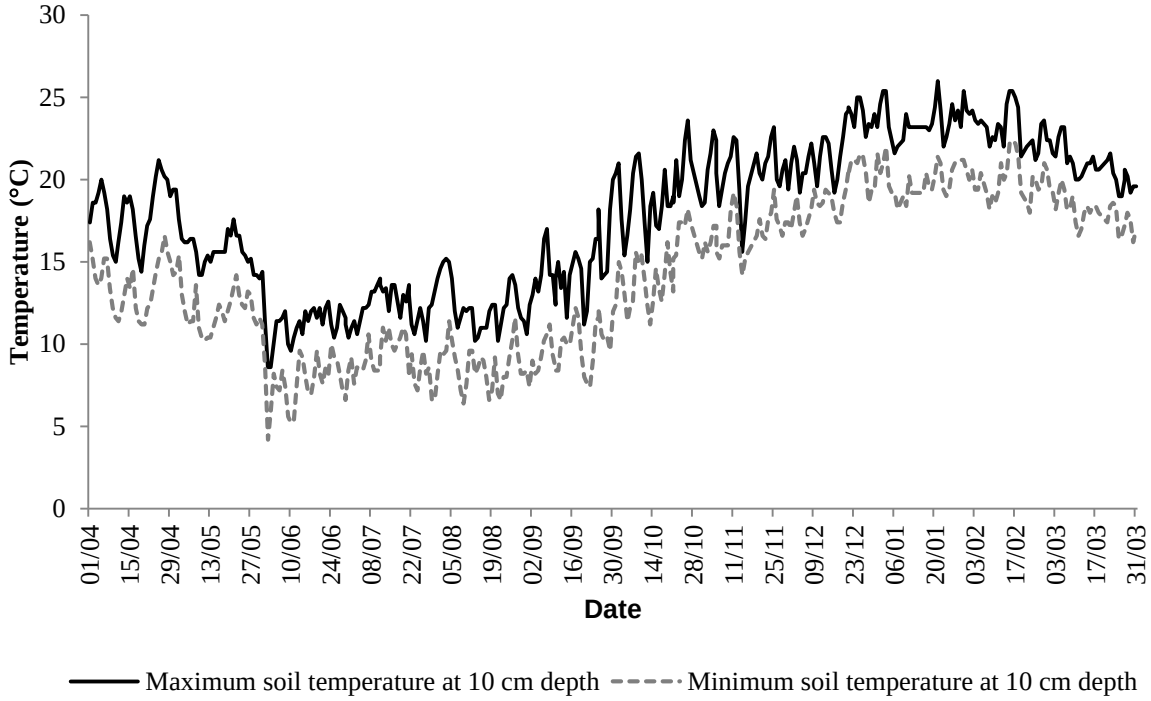

Fig 1. Average daily maximum and minimum soil temperature (°C) at 10 cm depth in the mature, bearing 'Golden Delicious' (site 1) during the 2013/2014 season (01 April 2013 – 31 March 2014).

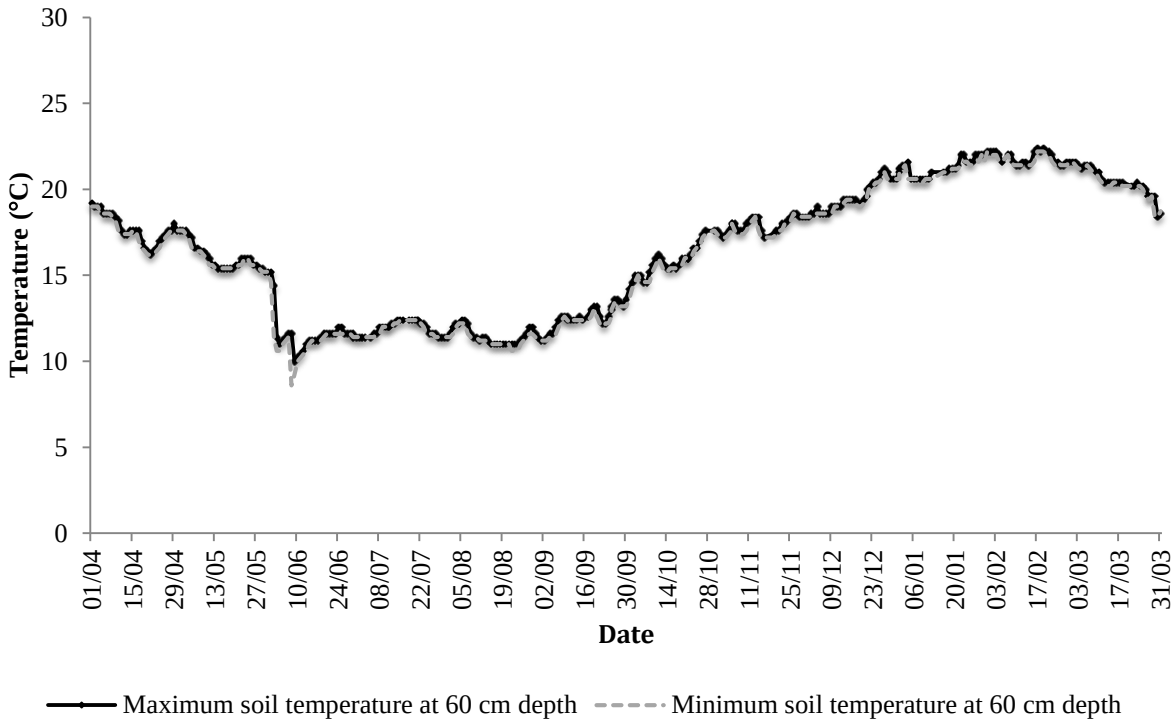

Fig 2. Average daily maximum and minimum soil temperature (°C) at 60 cm depth in the mature, bearing 'Golden Delicious' (site 1) during the 2013/2014 season (01 April 2013 – 31 March 2014).

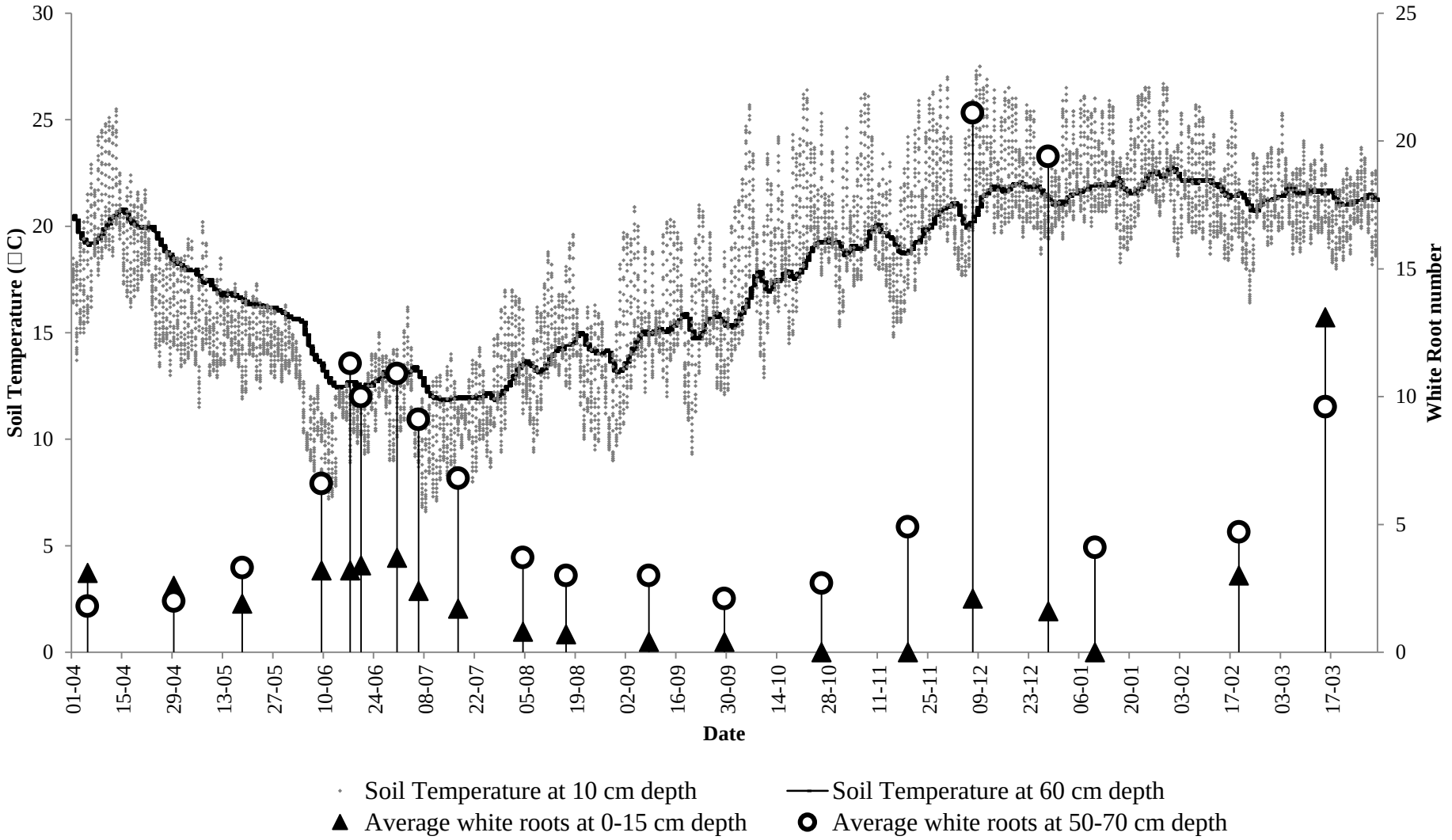

Fig 3. The variation in soil temperature (°C) at 10 and 60 cm depth compared to the variation in average white root numbers at 0-15 cm and 50-70 cm soil depth as observed from 1 April 2014 until 30 March 2015 for mature 'Golden Delicious'\M793 (site 1).

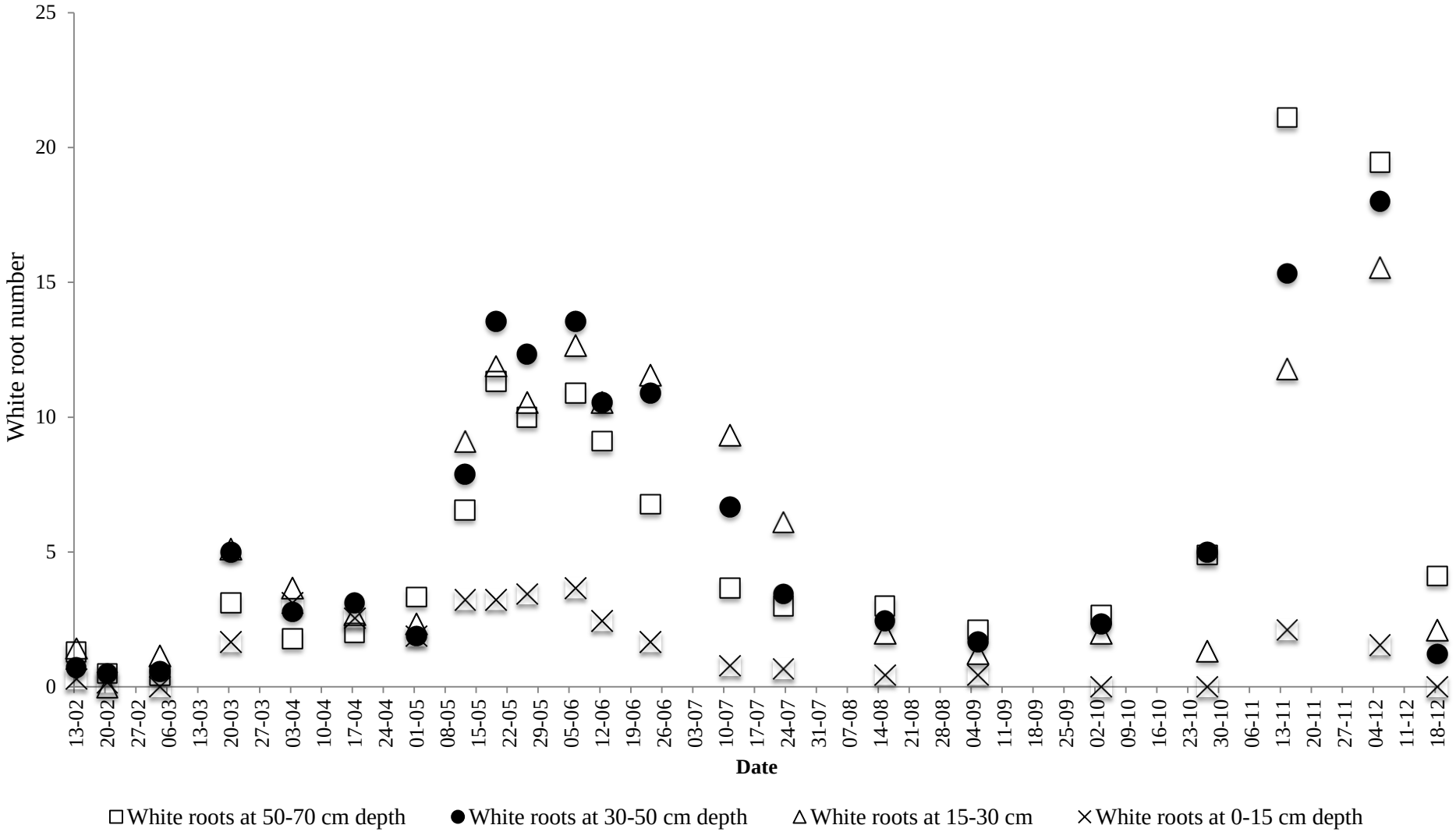

Fig 4. The seasonal variation in average white root numbers at four soil depths (minirhizotron windows) for mature bearing 'Golden Delicious' trees (site 1) from February until December 2014.

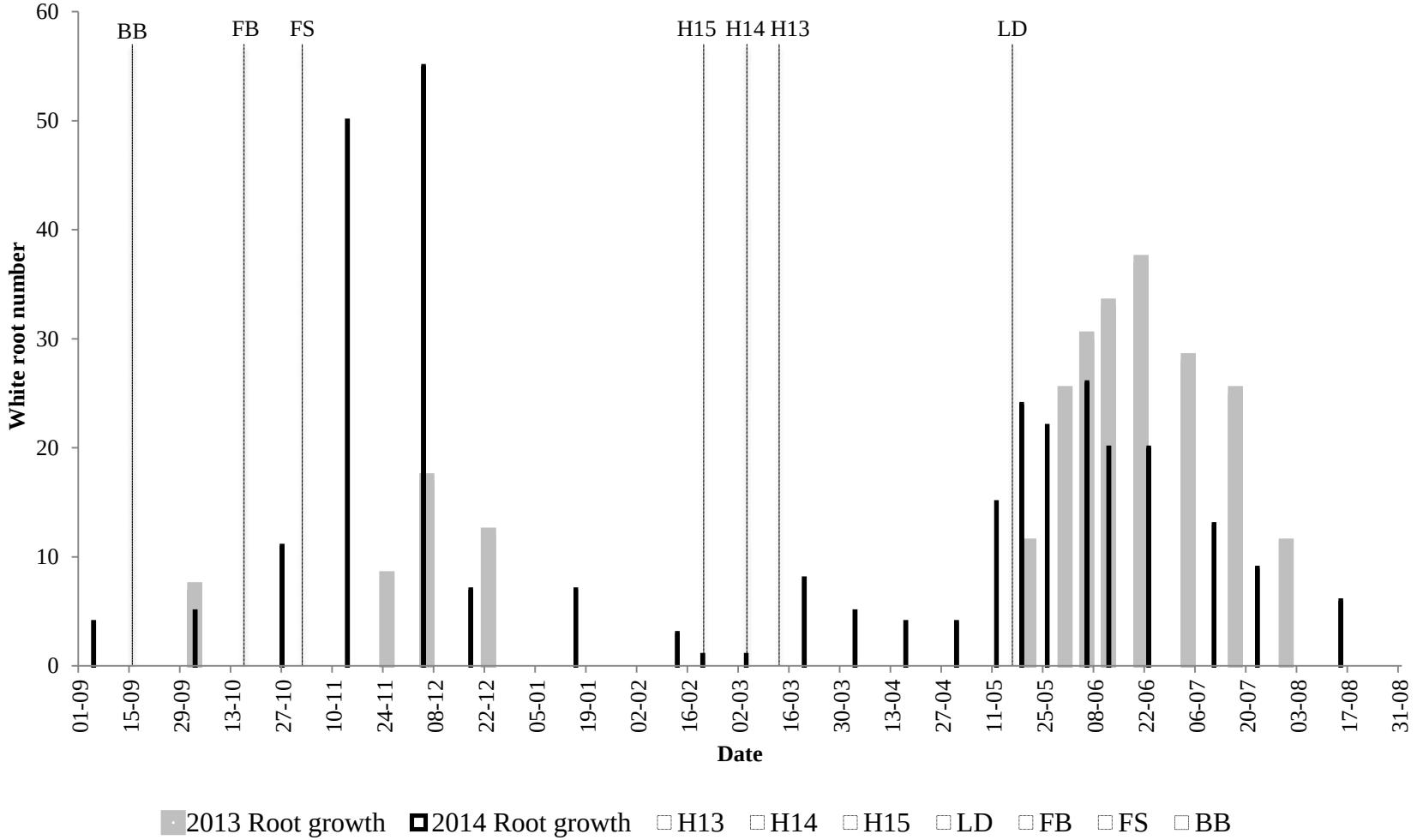

Fig 5. The variation in white root numbers in relation to phenological events, including bud break (BB), full bloom (FB), fruit set (FS), harvest – 2013 (H13), 2014 (H14), 2015 (H15) and 50 % leaf drop (LD) from 1 May 2013 until December 2014 for mature, bearing 'Golden Delicious' (site 1).

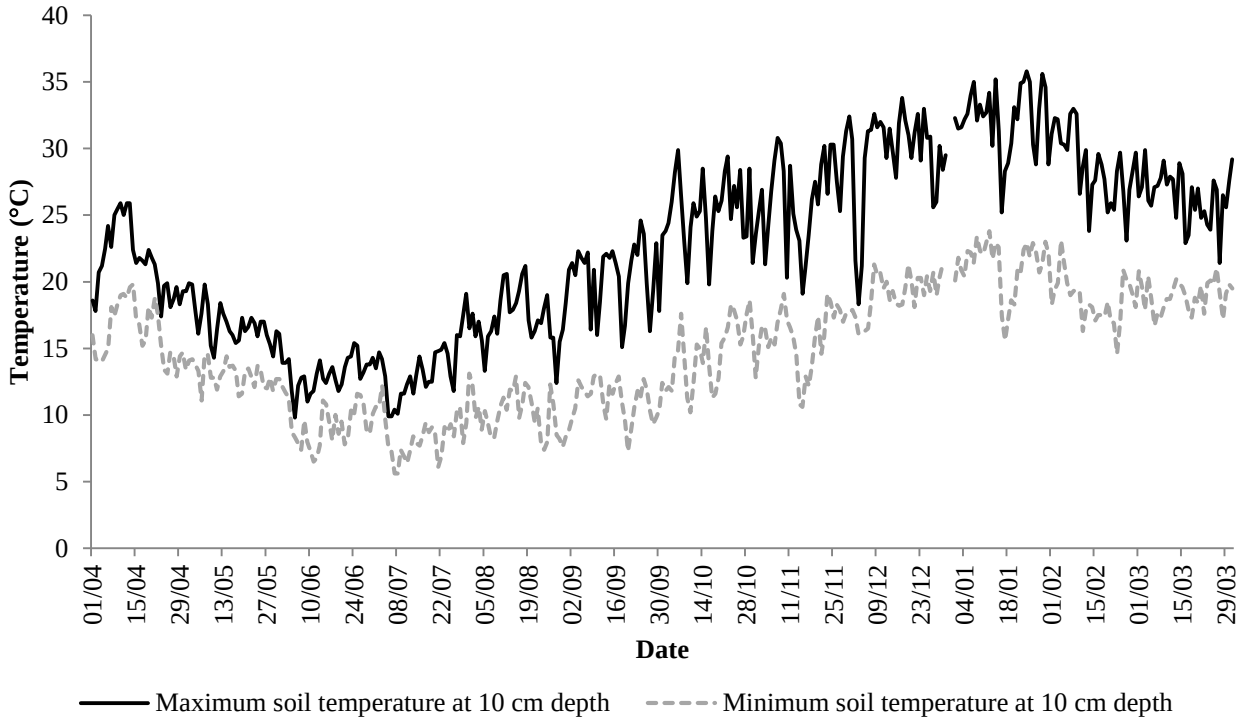

Fig 6. Average daily maximum and minimum soil temperature (°C) at 10 cm depth in the young non-bearing 'Corder Gala' site (2) during the 2013/2014 season (01 April 2013 – 31 March 2014).

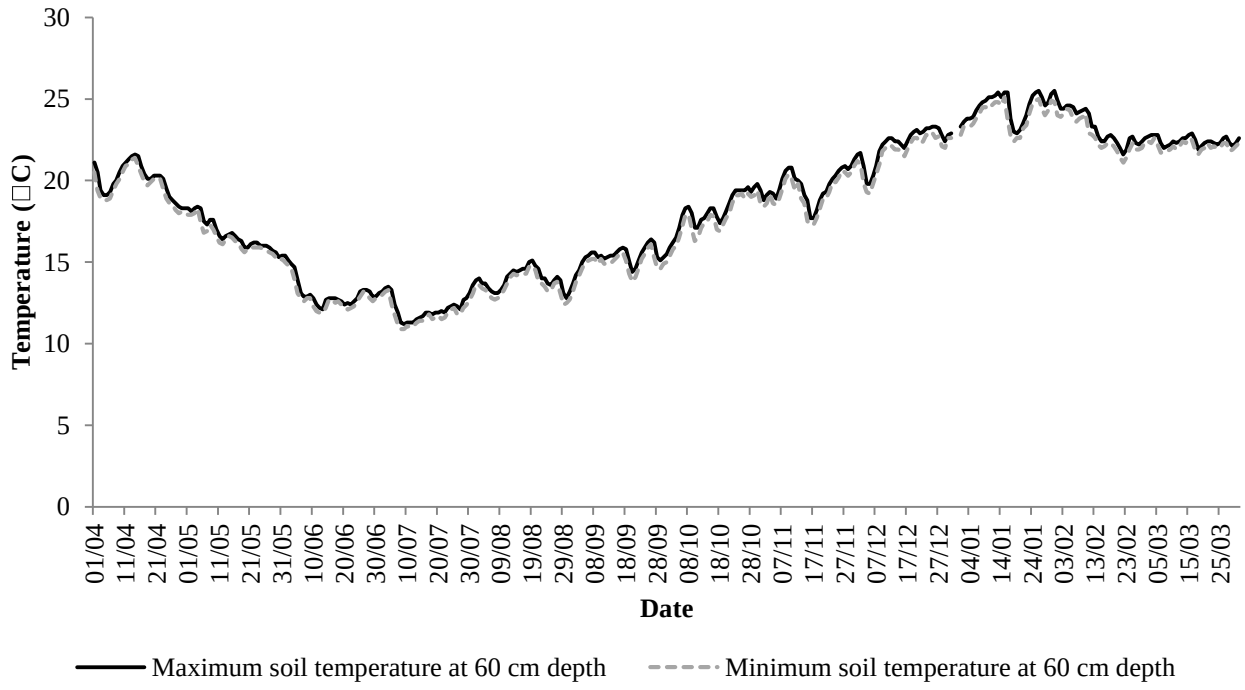

Fig 7. Average daily maximum and minimum soil temperature (°C) at 60 cm depth in the young non-bearing 'Corder Gala' site (2) during the 2013/2014 season (01 April 2013 – 31 March 2014).

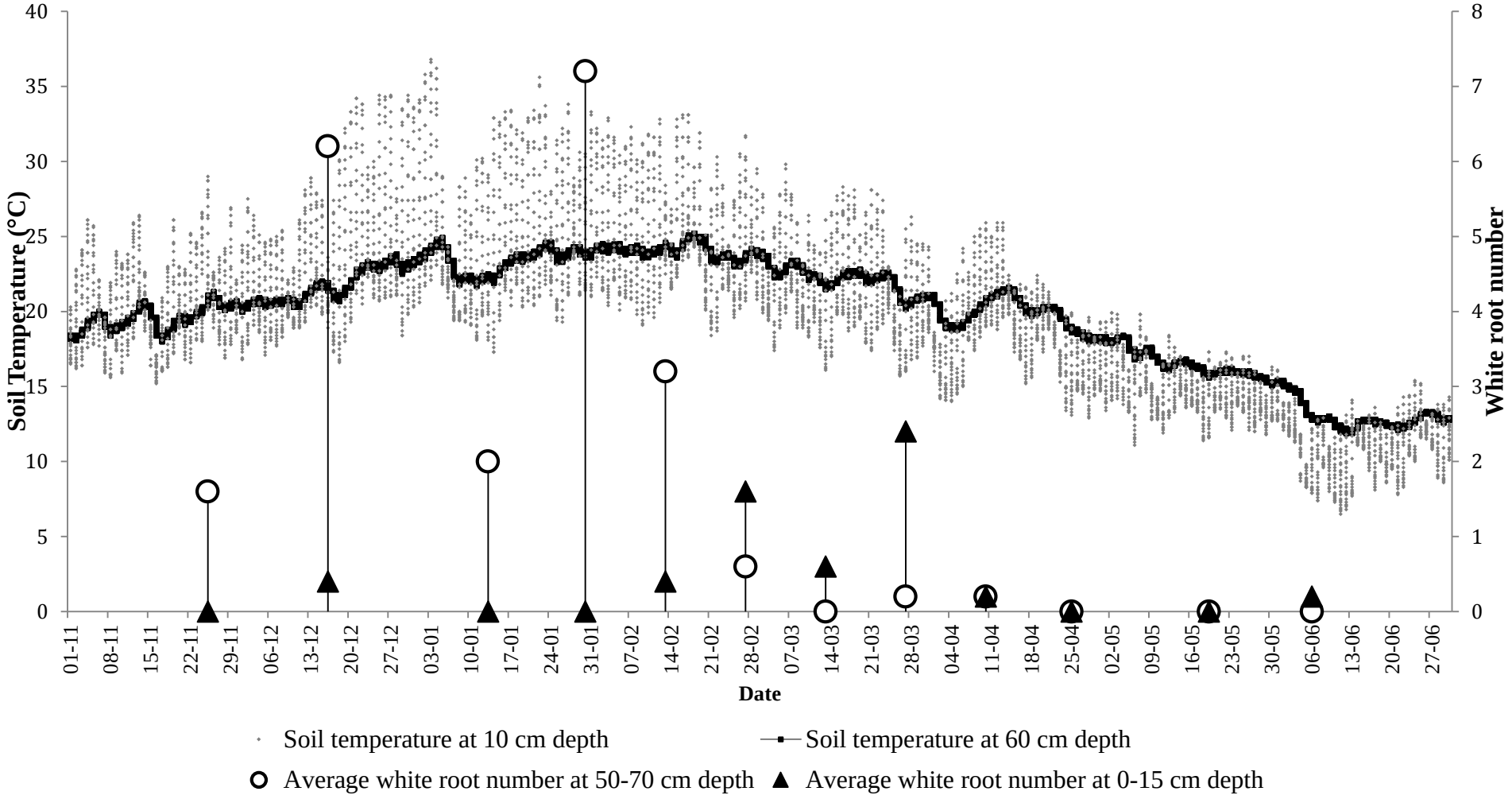

Fig 8. The variation in soil temperature (°C) at 10 and 60 cm depth compared to the vatiation in average white root numbers at 0-15 cm and 50-70 cm soil depth from 1 November 2013 until 30 June 2014 for the non-bearing 'Corder Gala'/M7 (site 2).

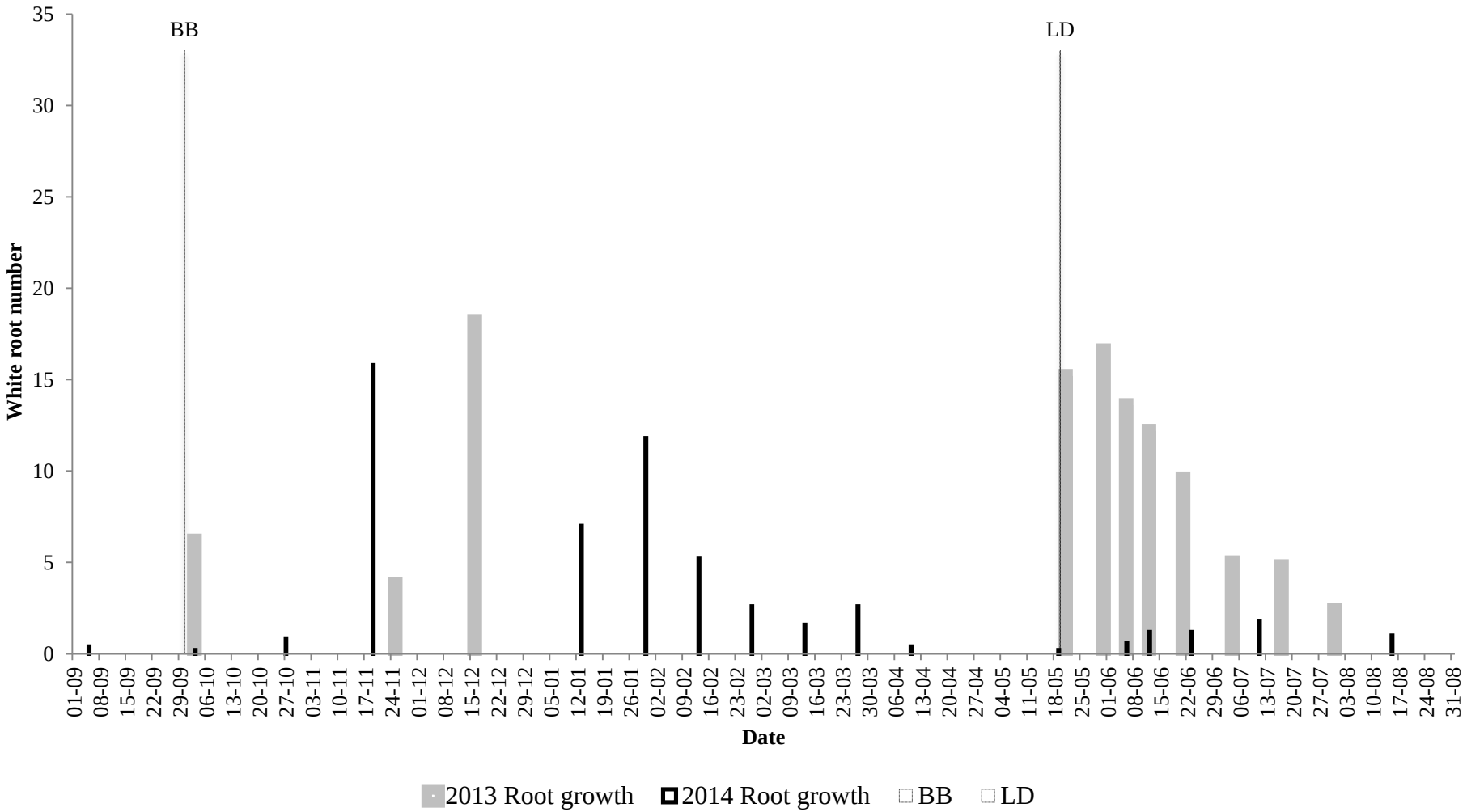

Fig 9. The variation in white root growth numbers in relation to phenological events, including bud break (BB) and 50 % leaf drop (LD) from May 2013 until November 2014 for young, non-bearing 'Corder Gala' (site 2).

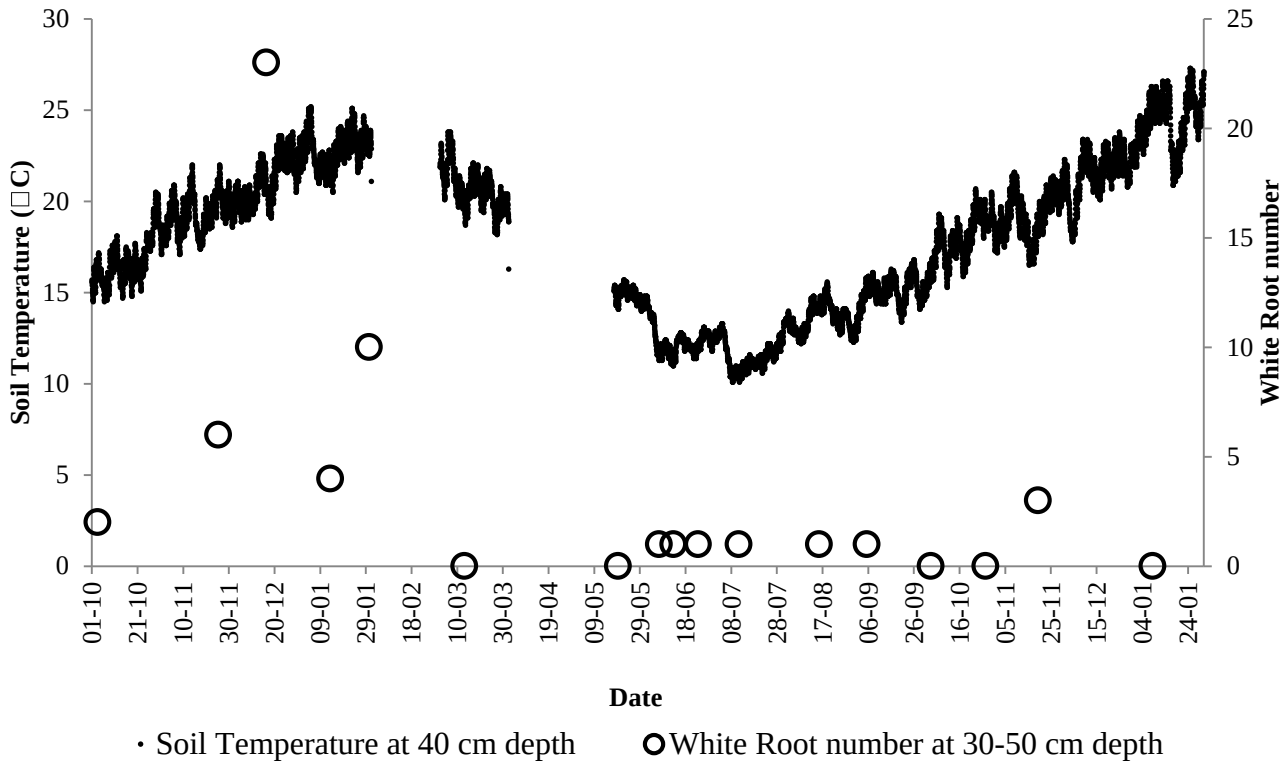

Fig 10. The variation in soil temperature (°C) at 40 cm depth compared to the variation in average white root numbers at 30-50 cm soil depth of a single non-bearing 'Corder Gala' tree from 1 October 2013 until 30 January 2015.

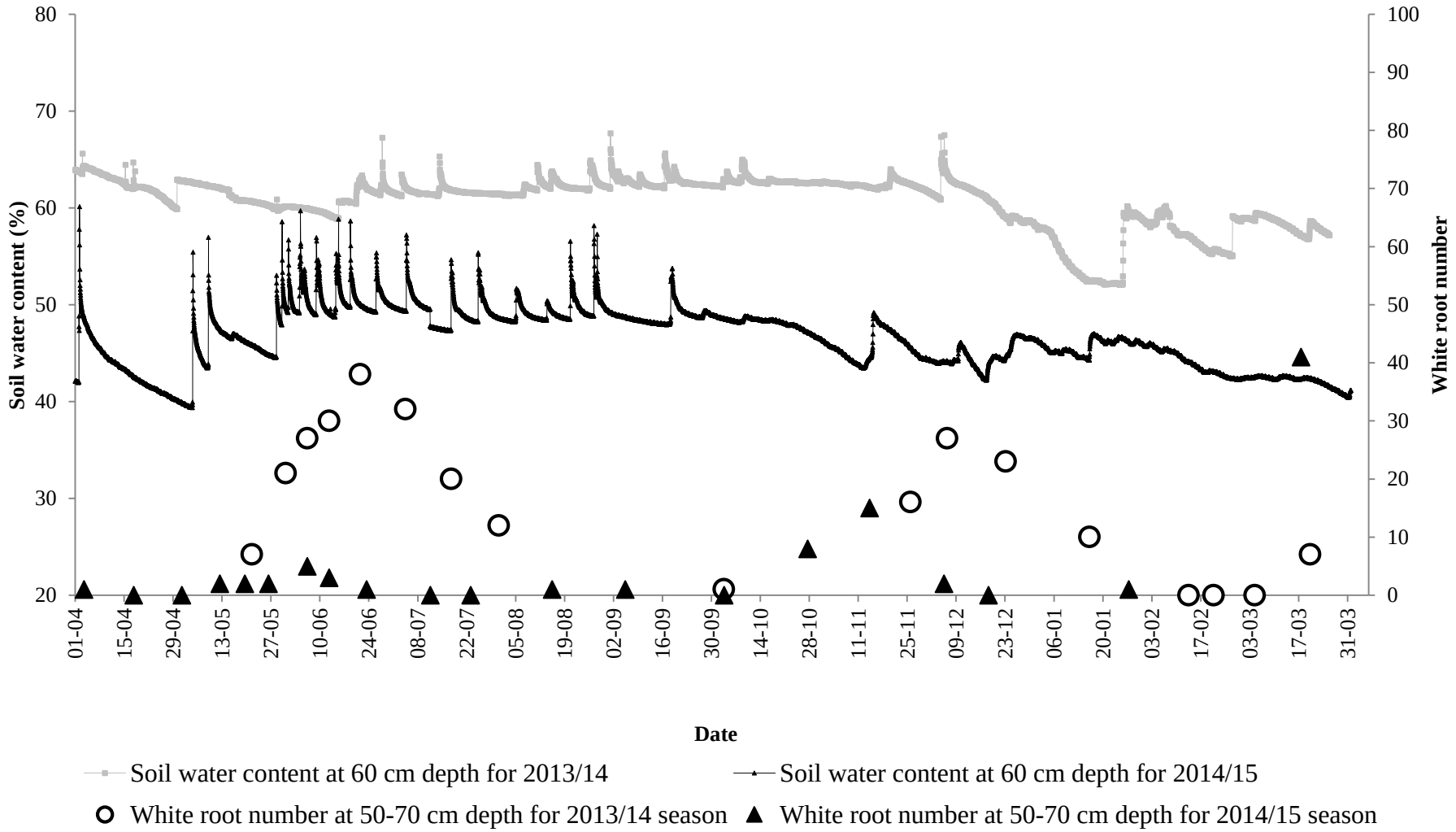

Fig 11. The variation in soil water content (%) at 60 cm depth compared to the variation in average white root numbers at 50-70 cm soil depth for two consecutive seasons for a single tree of the mature, bearing 'Golden Delicious' (site 1) from 1 April 2013/14 until 31 March 2014/15.

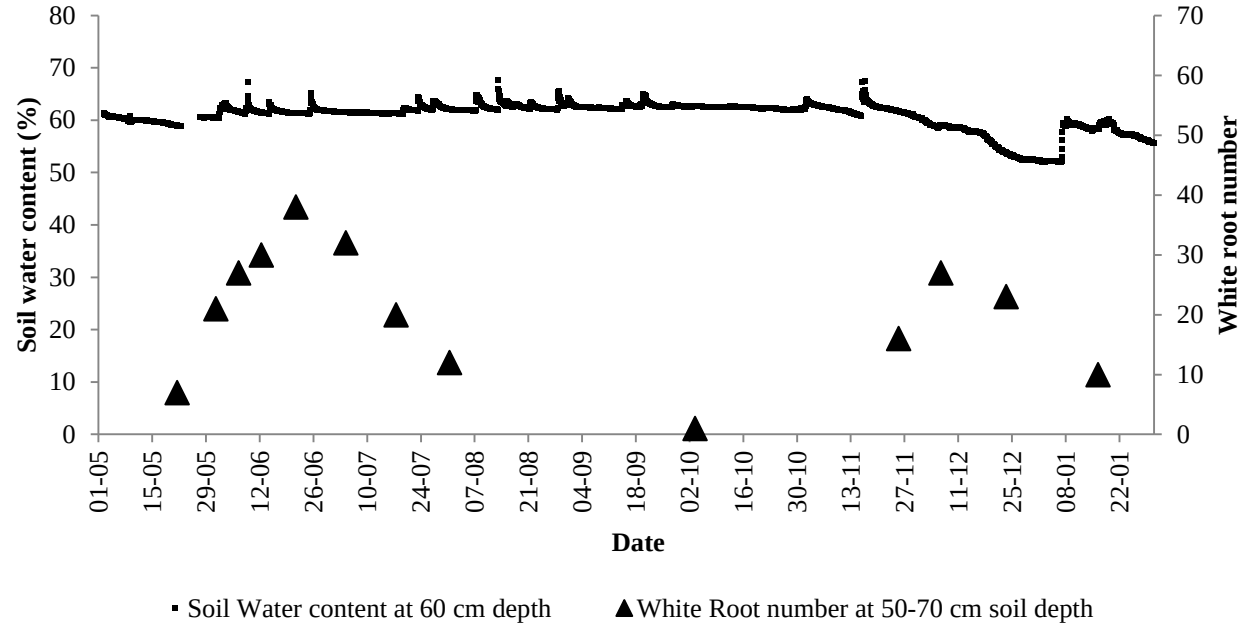

Fig 12 (A). The variation in soil water content (%) at 60 cm depth compared to the variation in white root numbers at 50-70 cm soil depth of a single mature bearing 'Golden Delicious' tree from 1 May 2013 until 30 January 2014.

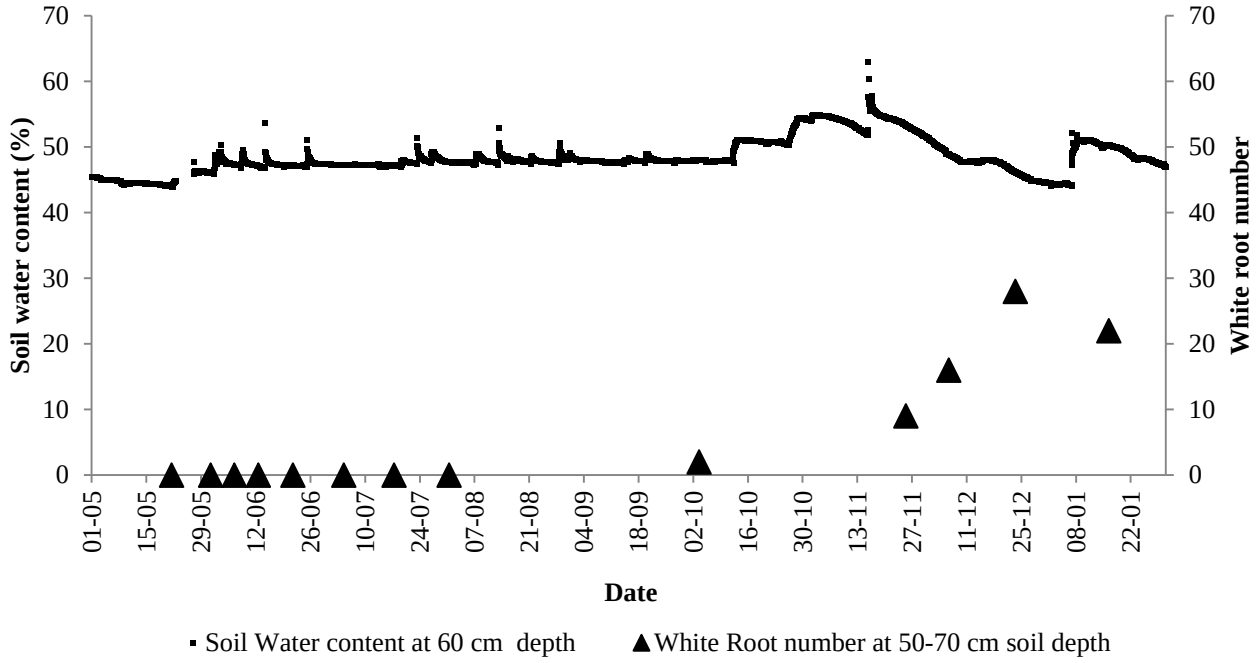

Fig 12 (B). The variation in soil water content (%) at 60 cm depth compared to the variation in white root numbers at 50-70 cm soil depth of a single mature bearing 'Golden Delicious' tree from 1 May 2013 until 30 January 2014.

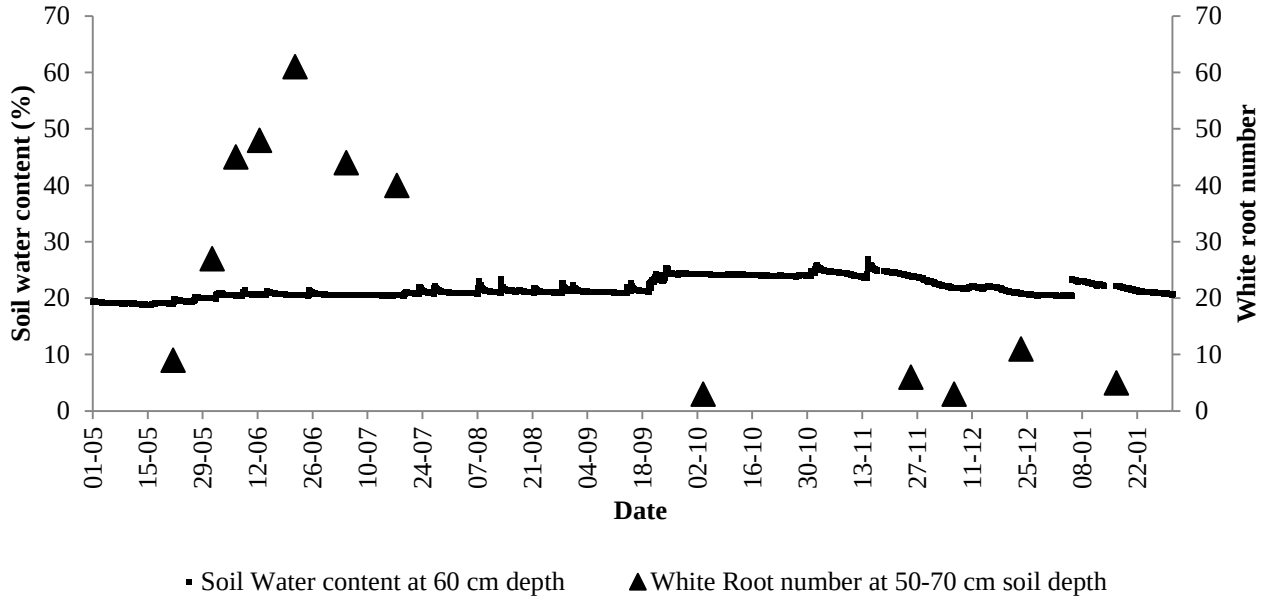

Fig 12 (C). The variation in soil water content (%) at 60 cm depth compared to the variation in white root numbers at 50-70 cm soil depth of a single mature bearing 'Golden Delicious' tree from 1 May 2013 until 30 January 2014.

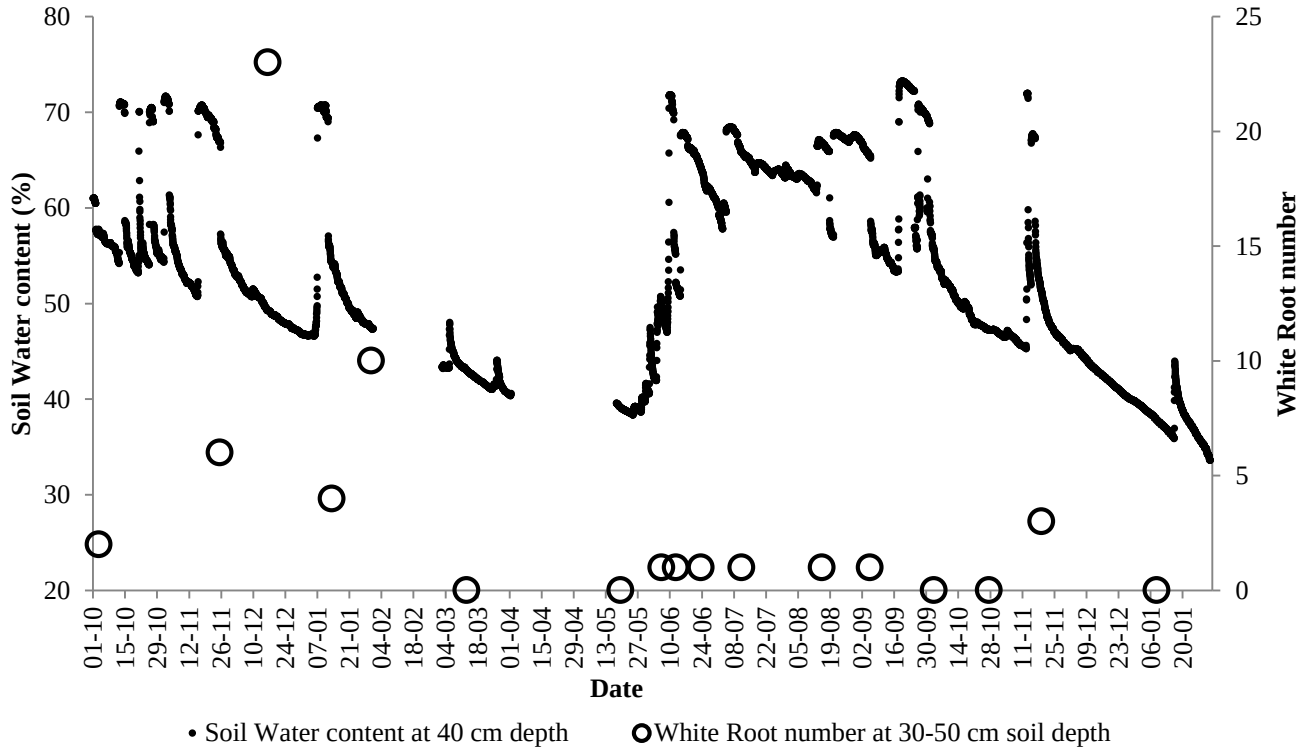

Fig 13. The variation in soil water content (%) at 40 cm depth compared to the variation in white root numbers at 30-50 cm soil depth of a single non-bearing 'Corder Gala' tree from 1 October 2013 until 30 January 2015.

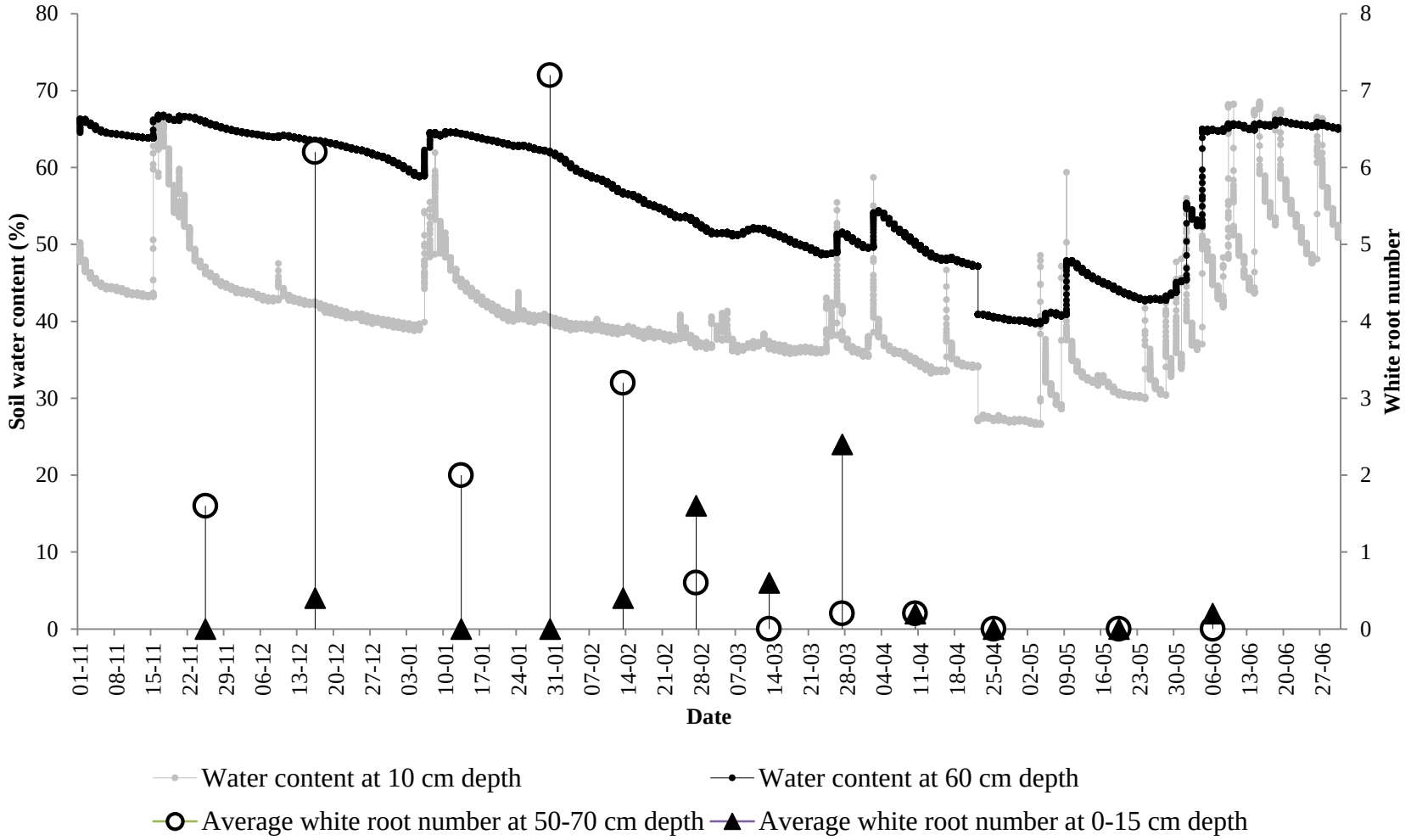

Fig 14. The variation in soil water content (%) at 10 and 60 cm depth compared to the variation in average white root numbers at 0-15 and 50-70 cm soil depth for young non-bearing 'Corder Gala' site (2) from 1 November 2013 until 30 June 2014.

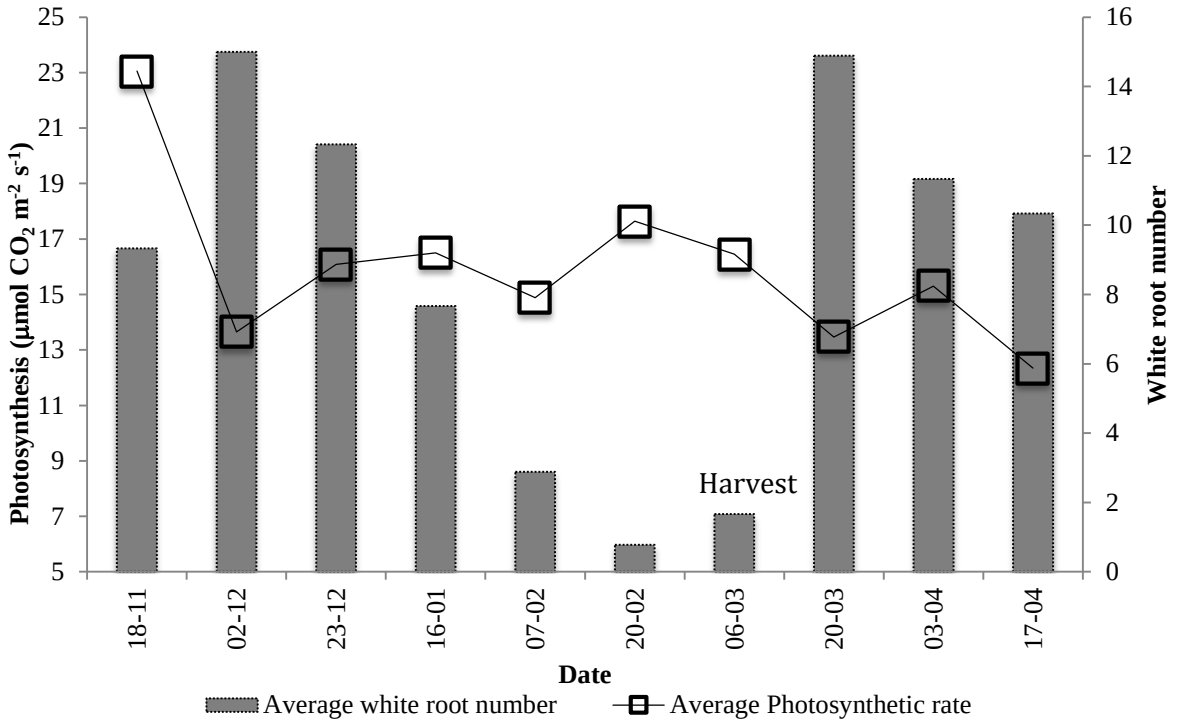

Fig 15. The variation in average photosynthetic rates (µmol CO_2 m^{-2} s^{-1}) compared to the variation in average white root numbers for mature bearing 'Golden Delicious' trees (site 1) from 25 November 2013 until 17 April 2014.

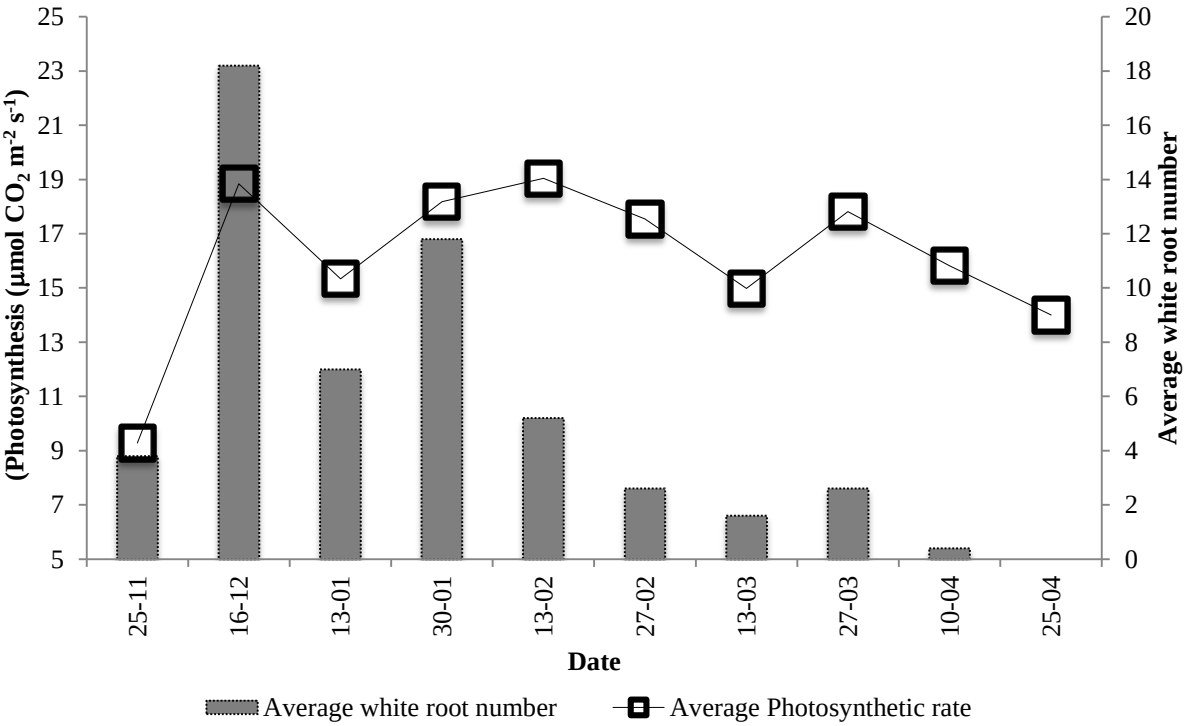

Fig 16. The variation in average photosynthetic rates (µmol CO_2 m^{-2} s^{-1}) compared to the variation in average white root numbers for young non-bearing 'Corder Gala' (site 2) from 25 November 2013 until 25 April 2014.

Paper 3

Evaluating the uptake of soil applied Calcium in relation to white root growth in apple trees.

Introduction

Calcium (Ca) dynamics in commercial horticultural fruiting plants are of particular interest, as adequate soil Ca and a healthy root system do not guarantee sufficient quantities of Ca in fruit tissues required for optimum quality (Saure, 2005; Vang-Petersen, 1980). Ca is therefore considered a 'problem nutrient' in fruit crops, as symptoms of suboptimal growth and physiological disorders arise as a consequence of inconsistent Ca supply to actively growing tissues. Ca translocation to developing apple fruit occurs primarily in the xylem and is influenced by the number and size of fruits, competition with shoot growth, transpiration rate and the difference in metabolic activity between fruit and other developing tissues (Saure, 2005; White and Broadly, 2003). Insufficient Ca levels in fruit, such as apple, make them more susceptible to physiological disorders, for example bitter pit, cork spot and Johnathan spot and also reduce storage time and shelf life of the fruit (Sharples, 1980). This is mainly due to the important role of Ca in cell wall integrity (Aghdam et al., 2012). Ca is furthermore important in secondary signaling, with a highly regulated, and consequently very low, cytosol concentration. Redistribution of Ca to actively growing tissues via the phloem cannot compensate for insufficient xylem supply, as Ca movement through the symplasm is limited (White and Broadley, 2003). The negative effect of inadequate or interrupted xylem flow to the tissue is particularly prominent in fruits with a high growth rate and a low transpiration rate (White and Broadly, 2003). The high regulation of Ca in the cytosol (symplast) and the abundance of appropriate binding sites in the intracellular matrix (apoplast) allow for a large proportion of total Ca to be located in the cell walls, distinguishing its distribution on a cellular and tissue level from other macro nutrients (Marschner, 1995).

White roots of fruit trees are known as absorbing roots and a shift in function occurs with root browning and suberization as roots mature and specialize more in storage and/or solute transport (Baldi et al., 2010; Ma et al., 2013; Vargas, 2015; White, 2001). Actively growing white roots have a particularly high potential for Ca uptake from the soil, especially at the root apex, elongation zone, as well as zones that remain non-suberized (white) following elongation

(Danjon et al., 2013, Marschner, 1995; Taiz and Zeiger, 2010; Tromp, 1980). The colour transition in roots, from white to brown due to pigmentation through tannin deposition, is associated with the maturation (suberin deposition and lignification) of the endodermis i.e. casparian band formation (Baldi et al., 2010; Kaspar and Bland; 1992; Ma et al., 2013; Nightingale, 1935). This causes a discontinuity in the apoplastic pathway from the root surface to the xylem, which is considered the primary pathway for Ca uptake into the plant. Congruent with Ca uptake rates along the root axis, anatomical studies of apple roots and the successive stages of endodermal casparian band development show a gradient in uptake potential for Ca along the axis of developing white roots (Marschner, 1995; Nightingale, 1935; White, 2001). Other alternative apoplastic pathways for solute movement into the xylem do, however, temporarily occur where lateral white roots emerge from the pericycle of older roots (White and Broadly, 2003). Apoplastic pathways into the stele may also occur as a result of small cracks in the endodermis of roots undergoing secondary growth (Zimmerman et al., 1971). Therefore, although new white roots have the highest potential for Ca uptake, brown roots may also contribute significantly to the total Ca uptake by the plant. New root production is periodic in bearing apple trees and the timing of phases depends on the dynamics of endogenous tree conditions, resulting from the interaction of the rootstock-scion combination with the particular climatic conditions (Ma et al., 2013; Psarras et al., 2000).

Destructive tissue sampling provides quantifiable information with regards to seasonal nutrient dynamics of apple trees (Cheng and Raba, 2009; Hanekom, 1973; Kangueehi, 2008; Terblanche, 1972). Annual uptake and distribution patterns of essential nutrient elements were quantified by Terblanche (1972) for two year old potted ‘Golden Delicious’/M793 trees in sand culture. Two main periods for active Ca uptake were observed through sequential whole-tree tissue analysis. From bud break, Ca uptake slowly increased, reaching a peak during the shoot extension phase (late spring to early summer), followed by lower uptake rates until harvest. A second, relatively high peak in Ca uptake rate commenced after harvest and continued until leaf drop in winter. However, the effect of applying additional Ca applications at different concentrations and seasonal timings on the uptake efficacy and distribution were not investigated in the study by Terblanche (1972) and was also not related to white root activity.

An increase of Ca in new growth of bearing apple trees is reported to commence around six weeks after bud break (Kangueehi, 2008). This overlaps with the time when the highest Ca concentration is found in young fruit, during the period of rapid cell division (Bergh, 1990;

Miqueloto et al., 2014; Saure, 2005). Actively growing white roots, considered to be very important for Ca uptake from the soil, are not always present during this critical period of fruit Ca accumulation (Eissenstat et al., 2006; Kangueehi, 2008; Psarras et al., 2000). Therefore, Ca supply to the fruit during this critical fruit growth stage may primarily be dependent on reserves in the tree. Calcium reserves are therefore of major importance to fruit quality, especially in more mature orchards where active root growth is absent/limited between bud break and fruit set (Ferguson, 1980).

Ca reserves are readily mobilized from the wood and bark early in the season, thereby increasing xylem Ca concentration significantly before leaf emergence (Ferguson, 1980; Saure, 2005; Terblanche, 1972), as well as making a substantial contribution to the Ca content of the shoots, leaves and fruit during the rapid shoot extension phase (Terblanche et al., 1979). The first few weeks after bloom are considered to be very important for Ca transport to the new apple fruitlets that quickly start to compete with the demand for Ca by vegetative growth (Saure, 2005). Ca is predominantly transported into the fruit via xylem vascular bundles, distinguishing it from most other mineral elements that are transported to the fruit via the phloem (Miqueloto et al., 2014). However, in contrast to the phloem, primary vascular bundles (xylem) supplying Ca to the fruit systematically disintegrate as the fruit cortical tissue expands, resulting in decreased fruit Ca uptake as the season progresses (Miqueloto et al., 2014). Furthermore, the primary vascular bundles of cultivars that are more susceptible to Ca related disorders tend to disintegrate at a faster rate (Miqueloto et al., 2014). Thus, optimizing fruit Ca intake, while primary vascular bundles are fully functional should be an important aim for apple growers managing orchards susceptible to Ca disorders. Although white root growth flushes may not overlap with the most important period of fruit Ca uptake, the potentially higher root Ca uptake rates during a root flush must surely be important in optimizing Ca reserve replenishment. As a result Ca uptake might be increased if additional Ca is applied during root flushes.

Commercially, soil application of fertilizers are often based on calendar dates and/or above ground phenological phases, with little regard to root growth dynamics (Eissenstat et al., 2006). Furthermore, supplemental soil applied Ca after establishment of an orchard is still not a common practice in South African apple orchards (Hanekom, 1973; J. Cronje, personal communication). Foliar applications of Ca are primarily implemented to increase fruit Ca content to acceptable levels in orchards showing physiological disordes caused by localized

fruit Ca deficiency (Lötze and Theron, 2003, 2006). There is however still scope to improve Ca levels of fruit trees through improved efficiency of soil applied Ca fertilizers, by ensuring synchronization of applications with white root growth dynamics (Wilsdorf, 2011). However, studies investigating the effect on Ca uptake after applying additional soil Ca fertilizer during white root flushes is scarce.

The objectives of this study was i) to determine if Ca uptake can be increased substantially by applying additional $Ca(NO_3)_2$ as soil application during periods of active white root growth in mature bearing apple trees and ii), to quantify the effect of additional Ca on uptake and distribution within one-year old potted apple trees. Ca uptake was quantified using leaf and fruit mineral analyses, fruit quality and yield (mature orchard) as well as Ca tissue distribution and concentration (potted trees) through destructive tissue analysis.

Materials and Methods

Pot trial

Experimental layout

The glasshouse was located on Stellenbosch University's Welgevallen Experimental Farm (33°56'49"S 18°52'16"E), Stellenbosch. Plant material consisted of small, one-year-old nursery trees ('Golden Delicious' on M7 rootstock) obtained from Stargrow Nursery (Stellenbosch, South Africa) at the end of winter (August, 2013). Trees were subjected to cold treatment at -0.5°C for 1 month to ensure uniform rest breaking. Before planting, individual trees were weighed, the height above the roots determined and the trunk diameter recorded 5 cm above the roots. The pots were lined with a transparent plastic bag before the medium was added to allow for the tree to be lifted later in the trial for observations of white root activity. On 17 September 2013, after sufficient winter chilling was accumulated, trees were planted in 5 L brown plastic pots, using only coarse filter sand as a growth medium. Roots were pruned off to a size suitable for planting into the pots and trees were topped, removing 18 cm from the apex.

The trial layout was a randomized complete block design where nine treatments were replicated, each on nine individual trees. Treatments comprised soil applications of $Ca(NO_3)_2$ at two different rates (1X, 3X) and a control (no additional Ca), which were applied either in summer, autumn or both summer & autumn. The standard industry recommendation (personal communication, Frank van den Heever, Yara Cape) for commercial application amounted to a $Ca(NO_3)_2$ (Yara Liva Nitrabor, Yara Africa Fertilizer (Pty) Ltd) treatment of 8 $g.pot^{-1}$ (1X) that was split into two applications, applied a week apart (Table 1). The second $Ca(NO_3)_2$ treatment (24 $g.pot^{-1}$) represented a 3X Ca rate and was split into three equal applications, applied with weekly intervals (Table 1). The $Ca(NO_3)_2$ was applied as granules to the surface of the pots and watered afterwards to ensure the Ca dissolved. The control treatment only received the Ca equivalent of half the concentration used in the Long Ashton nutrient solution which was applied to all plants.

All plants received the same balanced nutrient solution using fertigation throughout the trail. Initially, the Long Ashton (Hewitt, 1966) nutrient solution (EC = 2.3 $mS.cm^{-1}$) was applied, but with only 50 percent of the recommended concentration of Ca which amounted to 294 $mg.L^{-1}$ $CaCl_2.2H_2O$. From 24 January 2014 the Long Ashton nutrient solution was replaced by Mulifeed Classic (Nulandis, Stellenbosch) due to logistics, although the same amount of $CaCl_2$ was added to the nutrient solution as Multifeed does not contain Ca. The Multifeed and $CaCl_2$ (294 $mg.L^{-1}$) amounted to a final solution concentration with an EC of 2.3 $mS.cm^{-1}$.

Just after planting, in addition to the dripper schedule, trees were watered with approximately 0.2 L using a watering hose, to ensure establishment of the roots, as the sand medium tended to dry out quickly. Fertigation was applied with an automatically controlled drip system. The delivery rate of the drippers (Netafim) was 2 $L.h^{-1}.pot^{-1}$. Fertigation started on 18 October 2013 (one month after planting) and was applied 4 times per day for 2 min (0.27 $L.day^{-1}.pot^{-1}$) until early summer (17 December 2013), when fertigation was increased to 3 min cycles 4 times per day (0.4 $L.day^{-1}.pot^{-1}$). The duration of each cycle was set so that minimal runoff occurred.

Treatments were applied to coincide with established Ca uptake periods for young, potted trees as quantified by Terblanche (1972). White root activity was confirmed by visible observation of root growth through the inner transparent plant bag, after removal from the brown plastic pot, before administering treatments.

Plant Tissue and Leaf analysis

After fresh mass (FM) was recorded for all trees at planting, ten individual trees were randomly chosen and separated into roots and trunks and used for destructive macro mineral analyses by Bemlab (Pty) Ltd. (Strand).

At the end of April 2014, three weeks after the last autumn treatments, all plants were harvested. Individual tree height was determined from the base of the trunk, above the roots, to the apex of the tallest shoot. Trunk diameter was measured with a Vernier caliper 5 cm above the base roots. Thereafter each tree was divided into roots, trunk and new growth (shoots and leaves) to record the FM. After samples were dried, dry mass (DM) was recorded and sent to Bemlab (Pty) Ltd. (Strand) to determine the respective Ca concentrations. Ca concentrations (%) are therefore reported on a DM basis.

Soil Temperature

Soil temperatures in three containers, representing three levels of Ca application were recorded during the season using Tinytag data loggers (TGP-4505) (Gemini Data Loggers Ltd, Chichester, West Sussex, UK). Hourly temperature readings were recorded at a soil depth of approximately 15 cm from 20 September 2013 to 25 April 2014 (Fig. 1).

Field trial

Experimental site

Mature, full-bearing ‘Golden Delicious’ trees on M793 rootstock were selected in an orchard on a commercial farm, Applegarth (S 34° 08’10.2” E 019° 02’04.4” S), in Grabouw. The orchard was established in 2003 on a clay loam soil, with a 50% stone fraction. The trees were trained as central leaders and planted 2 m x 4.5 m. Standard commercial cultural practices were followed, including no additional soil Ca applications. Water was supplied via micro-jets, where a single emitter was positioned between two trees. The irrigation schedule was determined (ad hoc) by the farm management using evapotranspiration data. Standard annual fertilization was applied in granular form after harvest (220 kg/ha KAN). Weed control consisted of mowing the work row during early summer (December) and application of herbicides within the planting row around early January after mowing. Weeds in the tree row therefore grew abundantly during spring and early summer.

Experimental layout

The trail layout was a randomized complete block design, comprising three treatments replicated nine times, using two tree plots with buffer trees between the blocks. The treatments consisted of i) a control with no soil applied Ca and ii) soil applied $Ca(NO_3)_2$ (Yara Liva Nitrabor, Yara Africa Fertilizer (Pty) Ltd) applied either according to above-ground phenological stages (Industry) (commercial recommendation F van den Heever; Yara Western Cape) or iii) at visual confirmation of white root growth (Root flush)(according to MR root images). The application dates for the Industry and Root flush treatments are shown in Table 2. The treatment applications commenced with the post-harvest treatment in April 2013. The first postharvest Ca application of treatment 3 (visible white root growth), occurred two weeks after harvest (10 and 15 April 2013 (determined by excavation as the MR was not available yet). The same dosage of Nitrabor was used for both treatments, at the recommended level of 232 $g.tree^{-1}$ for each application time. However, at the beginning of the 2014/2015 season (7 November), the dosage was increased to 696 $g.tree^{-1}$ due to no increase in leaf or fruit Ca levels relative to the control under field conditions. Each treatment was split into two applications (about a week apart) for each of the application times. Granules were applied by hand around the base of tree trunk and within the range of the micro-jets.

Soil water content and Temperature

Soil water content and temperature dynamics were continuously measured at hourly intervals from September 2013, using wireless, capacitance based, continuous logging DFM Soil Moisture Probes (DFM Software Solutions CC, Penhill, South Africa) and Aquacheck Soil Moisture Probes (Aquacheck (Pty) Ltd, Durbanville, South Africa) soil probes. Data was recorded at six depths below the soil surface, 10 cm apart, to a depth of 60 cm.

White root growth dynamics

White root growth dynamics were determined using images obtained by MR technology, using a CI-600 Root Scanner (CID Bioscience, Camas, WA USA) and acrylic butyrate tubes of 1.05 m in length, with a diameter of 80 mm. Nine MR tubes were installed, monitoring three trees per treatment i.e. one tube per tree. Each tube was inserted approximately at a 45° angle and 40 cm from the trunk, parallel to the work row (Vamerali et al., 2012) on 15 April 2013. From

21 May 2013 images were collected bi-weekly until 31 July 2013, when white root growth declined, and less frequently from 3 October 2013 until 16 January 2014, when white root growth started again. Image collection on a weekly to bi-weekly basis continued from 13 February 2014 until 23 July 2014, when white root growth started and declined, after which images were collected less frequently until 17 March 2015. Root data was processed manually as discussed in Paper 1.

Shoot and Trunk growth

Annual one-year-old shoot growth and trunk circumference were recorded at the end of the 2012/2013 season on 12 June 2013 and the 2013/2014 season on 19 May 2014. For each plot, the length of new growth on 20 average shoots per block was recorded with a tape measure. Shoot sample distribution consisted of 10 shoots per tree, where two branches were selected on opposite sides of the tree and 5 shoots measured per branch. Trunk circumference was measured 20 cm above the ground for both trees, per plot and used to calculate yield efficiency.

Fruit quality, maturity and yield

At harvest, in both the 2013/2014 and 2014/2015 seasons, both trees per plot were stripped and all fruit weighed to determine the yield. A random subsample of approximately 100 fruit (17 kg) per plot was used for further evaluation. Ten fruit were used for maturity indexing at harvest at the Department of Horticultural Science, Stellenbosch University, for the following parameters: fruit size, fruit mass, firmness, starch breakdown, background colour and total soluble solids (TSS) according to standard procedures (Keithley, 1989). The remainder of the fruit were stored for 8 weeks at -0.5° C, followed by 2 weeks at 15 °C to initiate visual signs of possible defects, such as bitter pit. In the post storage evaluation, 20 fruit per plot were evaluated to determine background colour, firmness and sunburn incidence. The remainder of the fruit were inspected individually, only for visual defects such as bitter pit.

Fruit and Leaf mineral analysis

The macro mineral element concentrations of six fruit (similar size) per plot, randomly sampled at harvest, picked from both sides of the tree and both trees, was determined by Bemlab (Pty) Ltd. Leaf nutrient analysis was performed at the end of January each year according to industry

standards and analyzed for N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B. The leaf sample consisted of 20 leaves per plot, selecting five leaves from two opposite sides of the tree row, for each of the two trees per plot.

Soil analysis

A composite soil sample of four samples per plot was collected in June 2013 and 2014, at a depth of approximately 30 cm. Samples were analysed by Bemlab (Pty) Ltd. for both macro- and micro elements in 2013, but only for macro elements in 2014.

Soil solution analysis

Soil solution access tubes (SSAT) (Calafrica) were used for the extraction of soil solution in order to determine plant available Ca and NO_3^- after the application of treatments during 2013. The porous cups of the tubes were located at 30 cm soil depth. Soil solution extractions were performed approximately a week after application of treatments, within 24 hours after irrigation in the first season. However, the successful extraction of soil solution from all the tubes was only obtained for a single extraction date (1 November 2013) after the first industry treatment (25 October 2013), in spite of attempts afterwards at all following applications. The maximum negative pressure possible by the hand vacuum pump (75 kPa) was applied, after which the tubing was sealed with the clamp before detaching the vacuum pump from the tubing. After 3 hours enough soil solution was drawn into the cup for sampling. The clamp and the stopper were loosened and a 20 ml syringe was used to draw the solution up through the tubing. The sample was then placed in a labeled vial (20 ml) for analysis. The recommendation for best extractions was to coincide with soil water at field capacity, which was usually the day (for heavier soils) after an irrigation event for this particular model of SSAT. The concentrations of Ca and nitrate (mg/l) were determined with a reflectometer (RQflex 10, Merck KGaA, Germany) and appropriate reflectoquent strips. The average soil solution concentration (mg/l) of Ca and NO_3^- for 3 plots per treatment were used for comparing differences between treatments.

Statistical analyses

SAS (statistical analysis software) Enterprise Guide 5.1 (SAS Institute, Inc,, Cary, North Carolina, USA) was used to statistically evaluate differences between treatments using analysis of variance (ANOVA). Differences between treatments were significant at the 5 % level.

Results

Pot trial

Ca distribution in plant tissues

The Ca concentration in roots and new growth differed significantly among treatments, with no significant difference in trunk Ca concentration (Tabel 3). Both the standard (0.94 %) and higher (0.95 %) autumn application treatments, as well as treatments that received the standard concentration during both summer & autumn (0.97 %), accumulated significantly higher Ca concentrations in root tissues compared to the control (0.68 %) and the standard (0.67 %) summer application treatment. The high summer & autumn treatment also showed significantly higher root Ca concentrations than for the control and standard summer application treatment, but not from the high (0.74 %) summer application treatment. Roots showed higher Ca concentrations in treatments with autumn applications.

Ca concentrations in new growth showed significantly higher levels in the high summer & autumn application than the other treatments. The high summer application (0.97 %) did not differ significantly from the high summer & autumn application (1.04 %), but also not from the summer or summer & autumn treatments. The highest Ca concentrations in new growth were associated with treatments that included high summer applications. Autumn only treatments did not resulted in significantly higher Ca concentration of new growth compared to the control.

Vegetative growth

In Table 4, tree growth in terms of height and stem diameter is compared between treatments. No significances were reported for tree diameter, but significant differences were found for tree height although none were significantly different from the control.

There were no significant differences between treatments for either fresh (FM) or dry mass (DM) (Table 5). Root mass differed between treatments at $P<0.10$.

Soil temperature

Hourly temperatures of three pots were compared and plotted from September 2013, at planting, until harvest in April 2014. Average temperature values were used as temperatures between the three pots did not differ. The soil temperatures in the pots increased from September (planting) reaching maximum temperatures, above 35°C, during the summer months (November to February) (Fig. 1). Minimum temperatures during summer reached 16°C. A daily temperature fluctuation of 15°C was not uncommon in these small pots.

Field trial

Shoot growth

No significant differences between treatments were recorded for average shoot length for one-year-old shoots for either the 2012/13 or 2013/14 season. The average shoot length increased from the first to the second season (Table 6).

Yield

No significant differences occurred between treatments for yield or yield efficiency between treatments for either of the two seasons (Table 7). There was a decrease in yield and yield efficiency from the first to the second season after severe pruning, changing the tree shape/training system from a central leader to a solax during winter 2014.

Fruit maturity and quality

None of the fruit maturity parameters differed significantly among treatments, either at harvest (Table 8) or after storage in 2013/14 (Table 9). Fruit matured normally with a decrease in background colour, firmness and mass and an increase in TSS over time. Sunburn incidence showed no significant differences between treatments and no incidence of bitter pit was found.

During the second season, 2014/15 only TSS showed a significant difference between treatments (Table 10). Total soluble sugars were significantly higher for the root flush treatment compared to the industry treatment, but neither were significantly different from the control. Sunburn incidence was not recorded during this season. No incidence of bitter pit was found.

Average fruit mass was similar between seasons (approx. 160g), but average fruit diameter decreased from the first to the second season from approx. 73 to 60 mm (Tables 8 and 10).

Fruit and leaf mineral analysis

Fruit mineral analyses did not show any significant differences between treatments with regards to N, P, K, Ca and Mg content for either of the seasons (Table 11 and 12). Average fruit size did not differ significantly between treatments either, although average fruit size for mineral analysis were bigger (162g vs 136g) in the second season, that was accompanied with a slight decrease in fruit Ca concentration.

Leaf mineral analyses in the 2013/14 season showed no significant differences between treatments with regards to N, P, K, Ca, Mg, Na, Fe, Cu and B concentration. However, micro nutrient elements Zn and Mn showed significantly higher concentrations in the industry and root flush treatments compared to the control (Table 13). Similarly, no significant differences were found between treatments for leaf mineral analyses with regard to P, K, Ca, Mg, Na, Fe, Cu and B concentration during the second season, 2014/2015 (Table 14). There was a significantly lower N concentration in the industry treatment compared to the control and root flush treatment. There was also a slight increase (not significant) in leaf N concentrations from the first to the second season. In contrast, there was a slight increase in the leaf Ca concentration during the same period, with a tendency for the lowest Ca concentration in the industry treatment and the highest, in the root flush treatments.

Soil solution analysis

The soil solution extracted from the industry treatment plots were higher in both Ca^{2+} and NO_3^- following the industry treatment on 25 October 2013 (Fig. 2). The average Ca^{2+} concentration for the industry treatment was 199 mg/l compared to 103 and 95 mg/l for the control and root

flush treatments respectively. For NO_3^-, the average soil solution concentration for the industry treatment was 892 mg/l compared to 144 and 106 mg/l for the control and root flush treatments respectively.

Soil analysis

No significant differences were found in macro or micro mineral elements concentrations in the soil at the commencement of the trial 2012/13 (Table 15 a, b), or the following season, 2013/14 (Table 16).

Discussion

Pot trial

Ca distribution

Timing and concentration of additional soil $Ca(NO_3)_2$ applications affected Ca distribution and concentration within young, non-bearing apple trees, indicating uptake of added Ca under these conditions. The distribution of Ca in young, non-bearing apple trees confirmed results from Terblanche (1972) under similar conditions, but in his study, trees did not receive additional Ca. Trees in our study benefitted from additional Ca during both summer and autumn which agrees with similar results from Terblanche et al. (1979) who found that additional Ca applied to the growing medium during spring and autumn was actively absorbed in relation to the amount applied. Initial values from 10 trees analyzed at planting indicated the reserve status for Ca before the treatments commenced. The initial average Ca concentration of the root system and trunk was 0.43 and 0.47 % respectively (Table 3).

Significant differences in Ca % between treatments were found for root and shoot tissues, whereas the Ca concentration in the trunk was not affected by the treatments and stayed relatively stable. Trunk Ca concentrations at harvest were between 0.41 and 0.48 %, indicating a slight decrease in some cases compared to the initial trees before planting. In apple, approximately one third of the Ca in new growth can be supplied by the reserves in the bark (Kangueehi, 2008; Terblanche, 1972), which might explain the slight decrease in trunk Ca concentration at the end of the trial. The increase in trunk Ca concentration during leaf drop reported by Terblanche (1972) was not observed in this study and is attributed to the early

harvesting of the trees for analysis, as it pre-dated leaf drop, the time when redistribution of Ca from the root system to the trunk usually occurs (Terblanche, 1972). Results involving nutrient distribution by means of destructive tissue analysis will be relative to the time of sampling and therefore needs to be taken into consideration in future trials focusing on improving Ca reserves in the tree (Cheng and Raba, 2009).

The Ca concentration of the roots was particularly affected by the timing of $Ca(NO_3)_2$ applications, rather than by the level of Ca applied. Roots had a significantly higher Ca concentration (0.93 - 0.97 % compared to 0.67 – 0.74 %) for treatments that included an autumn application (Table 3) and is congruous with previous reports (Terblanche et al., 1979). Nutrient uptake and translocation mainly favours a mineral increase in the roots of apple trees prior to leaf fall in autumn (Kangueehi, 2008; Ludders, 1980; Terblanche, 1972), as minimal above-ground nutrient translocation occurs during autumn (Ludders, 1980). The increase in root Ca reserves from 0.43 (initial concentration) to 0.68 % (control trees) by the end of the season can be partly explained by the substantial increase in fine roots (Stassen, 1980; Terblanche, 1972). Fine roots typically have higher nutrient concentrations compared to coarse roots (Dornbush et al., 2002; Gordon and Jackson, 2000), of which the initial root system mainly consisted of, whereas at the end of the trial fine roots made up the majority of the root system. The significantly higher root Ca (0.93 - 0.97 %) reserve status of trees that included an autumn treatment can be attributed only to the timing of additional Ca and not the different application rates (1X vs. 3X), as summer-only applications did not have a significant effect on root Ca %. Root system size did not seem to be the primary cause for/or result of differences in Ca uptake between the different autumn treatment rates, as neither fresh nor dry mass of roots differed significantly between treatments (Table 5). It is therefore unlikely that the higher root Ca % is associated with differences in fine root quantities, but indicates the effect of applying the recommended (1X) additional rate of $Ca(N0_3)_2$ during active root growth in autumn.

Early in the season, new growth acts as a strong sink for both Ca and N, contributing to an increase in above-ground dry matter (Cheng and Raba, 2009; Kangueehi, 2008). During the first few weeks after bud break, Ca reserves in the permanent plant tissues are a major source for new growth (Kangueehi, 2008; Stassen and Stadler, 1988). Calcium movement within the tree is favored towards tissues with a high metabolic activity, such as actively growing shoot, leaf and fruit tissues, as well as active meristems (Saure, 2005; Vang-Petersen, 1980). Acropetal Ca movement in the tree canopy is accelerated by transpiration. Shoot tissues are

therefore highly favoured during active growth early in summer in terms of sink demand for Ca, which was confirmed by higher Ca % in shoot tissue when Ca was applied in summer in this study. The high summer application of $Ca(NO_3)_2$ therefore seemed to have the greatest effect on increasing the shoot and leaf tissue Ca concentration and confirms previous reports that the greatest Ca increase in new growth occurs with the accumulation of dry matter during the season (Cheng and Raba, 2009; Terblanche, 1972). Previous studies have also shown that high nitrate availability in summer facilitates increased apple leaf Ca concentration (Ludders, 1980; Tromp, 1980; White, 2001), which confirms the results in our study.

The standard (1X) summer treatment did not have a significant increase in the Ca concentration of the shoots as compared to the control. Therefore, the Ca application rate in summer influenced the degree of Ca accumulation in the shoot tissues. Only high concentrations (3X) resulted in a significant difference compared to the control (Table 3). Ca accumulation in the shoot tissues compared to roots is therefore not only influenced by the timing of the application, but also the concentration or rate of Ca application. Root tissue Ca accumulation on the other hand was not significantly affected by the rate of application, indicating a higher root uptake efficacy as the standard application rate was adequate for a significant increase in root tissue Ca concentration. In contrast, a high application rate was required in summer to significantly increase tissue Ca concentrations of new growth. However, during autumn applications, a substantially higher root mass was present compared to the root system size that was present during summer applications. This is evident as newly grafted trees were planted in spring with sparse woody root systems with an average FM of 124 g compared to the average FM (520 g)(control trees) of the root system when autumn applications were made. In other words, additional brown roots (resulting from active root growth earlier in the season) accompanied the newly produced white roots in autumn which may have contributed to the higher Ca uptake efficacy of autumn applications. Although white roots have a higher potential for Ca uptake, brown roots still contribute to nutrient uptake (Ferguson, 1980; Zimmerman et al., 1971). The inability of the summer applications (1X) to increase shoot tissue Ca level could be attributed to the smaller root system at this time, with an associated lower specific root length. The shoot Ca concentrations of treatments receiving autumn applications only, were similar to the control as compared to treatments that received summer applications, indicating a lack of above-ground Ca translocation late in the season as shown by Terblanche (1972) and Hanekom (1973).

Regarding the effect of soil temperature on white root growth and Ca uptake, temperature conditions in this study may have negatively affected Ca uptake especially during summer, as the minimum temperature was 16 °C and the maximum temperature was 38 °C. High temperatures and/or a substantial fluctuations in soil temperature were reported to affect root morphology and physiology, which may lead to a higher rate of suberization and lignification of the endodermis, thereby decreasing the potential for Ca uptake (Marschner, 1995; Nightingale, 1935; Pregitzer et al., 2000; White, 2001). The drastic temperature fluctuation during summer may therefore have contributed to the insignificant difference between the standard (1X) summer treatment and the control.

Field trial

Shoot growth and yield

$Ca(NO_3)_2$ applications did not significantly affect shoot growth, yield or yield efficiency in neither the first or second season (Table 6 and 7). This contradicts findings where soil applications of $Ca(NO_3)_2$ in spring was associated with an increase in tree vigour and yield in young bearing 'Delicious' and 'Golden Delicious' apple trees (Raese and Staiff, 1990). According to Raese and Staiff (1990), higher applied concentrations (2 – 4 kg.tree^{-1}) of $Ca(NO_3)_2$ had the greatest effect, compared to lower rates (0.65 - 1.3 kg.tree^{-1}), although the increase in vigour and yield only became significant in the third year of applying treatments. In the current study, the recommended rates were much lower (0.23 kg.tree^{-1}) and may therefore have supplied insufficient N (to which the increased yield and vigor is attributed by Raese and Staiff, 1990) to increase yield and vigor in our study even though an increase in Ca^{2+} and NO_3^- concentration of the soil solution was detected after the application of the industry treatment (Fig. 2). In addition, the effect of Ca and/or N nutrition on yield would have been masked by the serious reduction in branches in the 2014/15 season due to the severe winter (2014) pruning, in which mainly thinning cuts were applied.

Fruit maturity and quality

The application of Ca during the root flush resulted in fruit with the highest TSS during harvest of the second season (2014/15), although it only differed significantly from the industry treatment and not the control (Table 10). This treatment also showed a higher (not significant)

starch breakdown compared to the other treatments, indicating a possible trend towards more advanced maturity. However, TSS differences were most likely coincidental, as fruit TSS is not usually associated with Ca concentrations in fruit. Fruit firmness, background colour, diameter and fruit mass were not significantly affected by the treatments. This shows no negative influence of the presence of N in the Ca formulation on fruit quality, but the expected beneficial effect of additional Ca on fruit quality is also not evident. The lack of response in fruit quality to soil $Ca(NO_3)_2$ applications was quantified in insignificant differences between treatments over the two seasons for fruit and leaf Ca as compared to the control (Tables 11, 12, 13 and 14). Fruit Ca concentration may have been diluted due to the bigger fruit sizes in the second season, which was attributed to the severe winter pruning which resulted in a fruit thinning effect (Hansen, 1987; Saure, 2005). This could also have contributed towards a lack of significant differences in fruit firmness in treatments receiving additional Ca in the 2014/15 season. Fruit firmness is generally associated with higher fruit Ca concentrations (Bangerth, 1979).

Fruit and leaf mineral analyses

In this study, $Ca(NO_3)_2$ treatments did not significantly affect fruit or leaf Ca concentrations. However, leaf Ca values for 2014 and 2015 range from 1.61- 1.76 % and 1.63 – 1.73 % respectively, indicating sufficient Ca levels when compared to the apple leaf Ca standards from Bemlab (Pty) Ltd., with low and high levels 1.47 and 1.96%, respectively. Thus, Ca applications to these trees should still be able to increase foliar Ca concentrations if sufficient additional Ca was taken up.

Our results regarding fruit Ca content did not agree with results found by Raese and Staiff (1990). In their study soil applied $Ca(NO_3)_2$ in spring for three consecutive years resulted in a higher fruit Ca concentration for both ‘Delicious’ and ‘Golden Delicious’ apple trees. However, soil $Ca(NO_3)_2$ applications by both Raese and Staiff (1990) and Wildsdorf (2011) only resulted in significantly higher fruit Ca concentrations after the third season of application. According to the mineral analyses of the soil and leaves, the mineral status of the trees in the current trial was satisfactory in both seasons, reducing the potential of any major effects from additional nutrient applications usually associated with treating deficiencies in crops. However, in soils with sufficiently high Ca levels, soil Ca applications tend to have a delayed effect on fruit Ca levels (Raese and Staiff, 1990; Van der Boon, 1980; Wildsdorf, 2011). In our study,

soil base saturation was around 70 % Ca, classifying it in the category of sufficient soil Ca, although an 80% Ca base saturation is suggested to be optimal (Terblanche, 1985). Furthermore, the effectiveness of soil applied Ca is particularly sensitive to soil type, irrigation efficacy, chemical formulation and concentration (Hanekom, 1973; Raese and Staiff, 1990; Van der Boon, 1980). The amount of $Ca(NO_3)_2$ applied in our study (232 g.tree^{-1}) was much lower than rates applied by Raese and Staiff (1990)(1312 g.tree^{-1}). In addition, apple trees on sandy soils – in the case reported by Raese and Staiff (1990) - generally respond better to soil applied Ca compared to the clay loam soil in our study (Van der Boon, 1980). Sandy soils have an inherently low adsorption capacity (i.e. low Ca status) and a tendency for an unsuitably high K – Ca ratio while a clay soil is characterized by a high adsorption capacity (Van der Boon, 1980). Therefore, it is possible that the delayed reaction of the soil applied Ca will only occur during 2015/16, as there is still scope for Ca absorption to clay through cation exchange. The higher rate of $Ca(NO_3)_2$ that was applied from November 2014, in conjunction with the predicted delayed response, may result in a fruit Ca increase with the 2015/16 crop.

The contribution of environmental factors to Ca uptake

Water scheduling and irrigation practices in the current field trial could have contributed towards the poor response of the trees to the soil applied Ca treatments. Although irrigation management and soil Ca status in this trial were adequate for achieving acceptable fruit Ca levels (Tables 11 and 12) of between 5.0- 5.4 mg.100g^{-1} FM (Sharples (1980) and Terblanche et al. (1980)), improved irrigation during summer could have potentially further increased tree, leaf and fruit Ca levels. Studies on Ca uptake in apple trees by Cheng and Raba (2009) and Hanekom (1973) suggest that optimal irrigation scheduling during summer is important for higher and/or continued fruit Ca accumulation until harvest. Seasonal Ca uptake patterns for apple differ from other nutrient elements, as Ca accumulation has the potential to continue until after dry matter accumulation ceases (Cheng and Raba, 2009). The highest fruit Ca concentration is reported to occur during the first few weeks after bloom (Miqueloto et al., 2014; Saure, 2005), coinciding with the rapid cell division phase (Bergh, 1990). However, the majority (61.7%) of fruit Ca accumulation is reported to occur from the end of shoot growth until harvest, which was attributed to well irrigated conditions during summer (Cheng and Raba, 2009). Soil water content in summer in this trial tended to be low in the upper soil layer (Paper 2, Table 1), which could have contributed towards sub-optimal uptake of soil applied Ca during application times. According to Hanekom (1973), under dry soil conditions, Ca

preferably accumulates in the wood at the expense of the leaves and fruit. We could not quantify this in our field study, as whole trees were not harvested.

In addition to soil water dynamics, ground cover characteristics could also influence the effect of soil applied fertilizer via competition for nutrients and water (Delver, 1980). In the field trial, abundant weed growth was observed during spring and early summer (Appendix, Fig. 6). Weed and grass roots were therefore prolific in the upper soil layer, as indicated in MR images at this depth. During this period, most white apple roots were observed deeper than 15 cm (Paper 2, Fig. 3 and 4), whilst numerous weed roots could be observed in the top 15 cm. The lack of new apple root production in the upper 15 cm during this period as well as competition from the abundant weed roots could therefore partly be the result of insufficient water and nutrient availability. Even though the weeds on the tree row were dead after the herbicide spray by mid summer (Appendix, Fig. 7), they could still obstruct the distribution area of the micro-jets if not monitored actively – resulting in irregular distribution of water. Efficient weed control could therefore decrease root competition as well as improve water distribution and availability for apple roots.

Temperature fluctuation (Paper 2, Fig. 1 and 3) in the 0-15 cm soil depth interval was also not conducive to apple root growth, further reducing the number of white roots required for Ca uptake in this region. Surface applications of Ca inherently result in restricted downward movement, especially in a clay soil where negatively charged exchange sites have a high affinity for divalent Ca ions (Korcak, 1980; Van der Boon, 1980). According to the soil solution analysis, much less Ca^{2+} was detected at 30 cm soil depth after an application of $Ca(NO_3)_2$ compared to NO_3^- (Fig. 2), indicating a reduced downward movement of Ca to deeper soil depths. Therefore, most of the Ca would be available for uptake in the root zone closest to the surface, where the trees in this trial could not utilize it due to insufficient white root numbers.

Finally, research has shown that trees growing with grass as a cover crop rather than under cultivation produce fruit with a lower N content (Sharples, 1980), as grass competes for N and water (Wiersum, 1980). Since nitrate has a positive effect on Ca uptake, competition from the grass and weeds for the applied nitrate could have contributed to lower Ca uptake by apple roots (Ludders, 1980).

Conclusion

Both the timing and concentration of soil applied $Ca(NO_3)_2$ influenced the uptake and distribution of Ca between the root system and new growth of young, non-bearing potted apple trees. The effect of application timing significantly influenced the Ca concentration of both the roots and new growth, whereas the effect of application rate only influenced the Ca concentration of the new aerial growth. Therefore, relatively high rates of $Ca(NO_3)_2$ applications in summer (3X) compared to the standard (1X) recommendation by Yara (F van den Heever) seemed to be necessary for increasing Ca concentrations in new vegetative growth for young establishing apple trees. However, during autumn, the roots system seemed to have a greater Ca acquisition efficacy, possibly due to the more developed root system as well as a more favourable soil environment in these pots during autumn, than in summer, regardless of application rate. The standard rate in autumn was sufficient to significantly increase root tissue Ca concentration, whilst higher rates had no additional affect. Summer-only applications did not affect root Ca concentrations and autumn applications did not affect the Ca concentration of the existing aerial growth – both findings agreeing with previous research in pots.

Under field conditions, the short term efficacy of additional $Ca(NO_3)_2$ applications on uptake in mature, bearing trees was not evident, either quantified by leaf or fruit nutrient analyses. However, the commercial application rate of $Ca(NO_3)_2$, was similar to the standard rate applied in the potted trial, where it also showed no significant effect on the Ca concentrations in new aerial growth. The concentration of applied $Ca(NO_3)_2$ may therefore have been too low to see any impact on tissue Ca. Another potential reason for the lack of any effect may have been the soil type, as the clay soil of the orchard has a high Ca adsorption capacity compared to the sand medium in the potted trial. A greater uptake response to soil applied Ca has been observed on a sandy compared to a clay soil (Van der Boon, 1980). Furthermore, previous results indicate a delayed effect of soil applied $Ca(NO_3)_2$ on the Ca concentration of new growth, as results only become significantly evident after two to three seasons of application (Raese and Staiff, 1990; Van der Boon, 1980; Wildsdorf, 2011). The uptake response of timing $Ca(NO_3)_2$ applications with active root growth, compared to the recommended calendar dates of application could, therefore not be determined after two seasons due to insignificant leaf and fruit response across treatments.

Therefore, the efficiency of applied $Ca(NO_3)_2$ (Nitrabor) in pots to increase Ca concentration of new growth was high if the recommended commercial rates are increased to 3X. If an

increase in leaf Ca concentrations is required in young establishing trees, the most efficient time for application is during active white root growth in summer. If an increase in the Ca concentration in the roots or possibly reserve tissues is the main aim, the recommended rate would suffice, but will only be efficient if applied during autumn root activity. The benefit of soil applied $Ca(NO_3)_2$ to increase Ca concentration in either fruit or reserve tissues still need to be confirmed under commercial orchard conditions in mature, bearing trees and could not be established in this trial. A further increase in application rate was applied during the third season for evaluation, but results were not available yet at the submission of this paper.

References

Aghdam, M. S., Hassanpouraghdam, M. B., Paliyath, G. and Farmani, B. 2012. The language of calcium in postharvest life of fruits, vegetables and flowers. *Scientia Horticulturae 144*, 102-115.

Baldi, E., Wells, C. E. and Marangoni, B. 2010. Nitrogen absorption and respiration in white and brown peach roots. *Journal of Plant Nutrition* 33, 461-469.

Bangerth, F. 1979. Calcium-related physiological disorders of plants. *Annual review of Phytopathology 17*(1), 97-122.

Bergh, O. 1990. Effect of time of hand-thinning on apple fruit size. *South African Journal of Plant and Soil 7*(1), 1-10.

Cheng, L. and Raba, R. 2009. Accumulation of macro-and micronutrients and nitrogen demand-supply relationship of 'Gala'/'Malling 26' apple trees grown in sand culture. *Journal of the American Society for Horticultural Science 134*(1), 3-13.

Danjon, F., Stokes, A. and Baker, M. R. 2013. Root systems of woody plants. Plant Roots The Hidden Half, (Ed) Eshel, A. and Beeckman, T. Taylor & Francis group, LLC. CRC press. Chapter 29 – 3.

Delver, P. 1980. Uptake of nutrients by trees grown in herbicide strips. In: Mineral nutrition of fruit trees. Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M. Studies in the Agricultural and Food sciences. Butterworths, London – Boston. p. 229- 240.

Dornbush, M. E., Isenhart, T. M. and Raich, J. W. 2002. Quantifying fine-root decomposition: an alternative to buried litterbags. *Ecology 83*(11), 2985-2990.

Eissenstat, D. M., Lakso, A. N. Neilsen, D., Neilsen, G. H. and Smart, D. R. 2006. Seasonal patterns of root growth in relation to shoot phenology in Grape and Apple. *Acta Horticulturae* 721, 21 -26.

Ferguson, I. B. 1980. Uptake and transport of calcium. In: Mineral nutrition of fruit trees (eds.) Atkinson, D., Jackson, J. E., Sharples, R. O. and Waller, W. M. Butterworths publishers p. 183 – 192.

Fumey, D., Lauri, P. É., Guédon, Y., Godin, C. and Costes, E. 2011. How young trees cope with removal of whole or parts of shoots: an analysis of local and distant responses to pruning in 1-year-old apple (Malus × domestica; Rosaceae) trees. *American Journal of Botany 98*(11), 1737-1751.

Gordon, W. S. and R. B. Jackson. 2000. Nutrient concentrations in fine roots. *Ecology* 81, 275–280.

Hanekom, A. N. 1973. Opname van kalsium-45 deur appel bome by verskillende vogpeile en die induksie van bitterpit. Ph.D tesis in Natuurwetenskappe, Randse Afrikaanse Universiteit. Suid-Afrika.

Hansen, P. 1987. Source-Sink Relations in Fruits I. Effects of Pruning in Apple/Assimilatverteilung bei Obstgehölzen I. Die Wirkungen des Schnittes beim Apfel. *Die Gartenbauwissenschaft 52*(5), 193-195.

Hewitt, E. J. 1966. Sand and water culture method used in the study of plant nutrition. Farnham Royal, England : Commonwealth Agricultural Bureaux.

Kangueehi, G. N. 2008. Nutrient requirement and distribution of intensively grown 'Brookfield Gala' apple trees. MSc thesis in the Department of Horticultural Science, Faculty of Agricultural Science, Stellenbosch University.

Kaspar, T. C. and Bland, W. L. 1992. Soil temperature and root growth. *Soil Science 154*(4), 290-299.

Keithley, M. 1989. Growers need to test their own apple maturity. Good fruit grower. March 15, p. 6 and 60.

Korcak, R. F. 1980. The importance of calcium and nitrogen source in fruit tree nutrition. In: Mineral nutrition of fruit trees. Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M. Studies in the Agricultural and Food sciences. Butterworths, London – Boston. p. 268

Lötze, E. and Theron, K.I. 2003. Bitterpit by Golden Delicious appels: 'n opname onder sagtevrugte produsente in die Wes-kaap. *South African Fruit Journal* Aug/Sep 15-18.

Lötze, E. and Theron, K. I. 2006. Dynamics of calcium uptake with pre-harvest sprays to reduce bitter pit in 'Golden Delicious'. *Acta Horticulturae* 721, 313-320.

Ludders, P. 1980. The effects of time and amount of nutrient additives on nutrient status and distribution and on fruit quality. In: Mineral nutrition of fruit trees. Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M. Studies in the Agricultural and Food sciences. Butterworths, London – Boston. p.166 -167.

Ma, L., Hou, C. W., Zhang, X. Z. Li, H. L., Han, De G., Wang, Y. and Han, Z. H. 2013. Seasonal growth and spatial distribution of Apple tree roots on different rootstocks or interstems. *Journal of American Society of Horticultural Science* 138(2), 79–87.

Marchner, H. 1995. *Mineral nutrition of higher plants second edition*. London. Academic press, p. 63-70, 285-299, 484-500, 508-513.

Miqueloto, A., do Amarante, C. V. T., Steffens, C. A., dos Santos, A. and Mitcham, E. 2014. Relationship between xylem functionality, calcium content and the incidence of bitter pit in apple fruit. *Scientia Horticulturae 165*, 319-323.

Nightingale, G. T. 1935. Effects of temperature on growth, anatomy, and metabolism of apple and peach roots. *Botanical Gazette* 96(4), 581-639.

Pregitzer, K. S., King, J. S., Burton, A. J. and Brown, S. E. 2000. Responses of tree fine roots to temperature. *New Phytologist 147*(1), 105-115.

Psarras, G., Merwin, I. A., Lakso, A. N. and Ray, J. A. 2000. Root growth phenology, root longevity, and rhizosphere respiration of field grown 'Mutsu' apple trees on 'Malling

9' rootstock. *Journal of the American Society for Horticultural Science 125*(5), 596-602.

Raese, J. T. and Staiff, D. C. 1990. Fruit calcium, quality and disorders of apples (*Malus domestica*) and pears (*Pyrus communis*) influenced by fertilizers. In: Plant Nutrition—Physiology and Applications. pp. 619-623. Springer Netherlands.

Saure, M. C. 2005. Calcium translocation to fleshy fruit: its mechanism and endogenous control. *Scientia Horticulturae 105*(1), 65-89.

Sharples, R.O. 1980. The influence of orchard nutrition on the storage quality of apples and pears grown in the United Kingdom. In: Mineral nutrition of fruit trees pp. 17- 19. Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M. Studies in the Agricultural and Food Sciences. Butterworths, London – Boston.

Stassen, P. J. C. 1980. A sudy on the carbohydrate and nitrogen metabolism in *Prunus persica*. Ph.D. Thesis, University of Stellenbosch, March, 1980.

Stassen, P. J. C. and Stadler, J. D. 1988. Seasonal uptake of phosphorus, potassium, calcium and magnesium by young peach trees. *South African Journal of Plant and Soil 5*(1), 19-23.

Taiz, L. and Zeiger, E. 2010. Plant Physiology, 5th Edition, Sinauer Associates, Inc. p. 1-34, 468-472.

Terblanche, J. H. 1972. Seisoensopname en verspreiding van tien voedings elemente by jong appel bome gekweek in sand kulture. Ph. D tesis in Landbou, Universiteit van Stellenbosch.

Terblanche, J. H. 1985. Integrated approach to fertilisation of apples for optimum production and quality under South African conditions. *Horticultural Science 3*, 1-6.

Terblanche, J. H., Gurgen, K. H. and Hesebeck, I. 1980. An integrated approach to orchard nutrition and bitter pit control. In: Mineral nutrition of fruit trees. pp. 71-82. Atkinson,

D., Jackson, J.E., Sharples, R.O. and Waller, W.M. Studies in the Agricultural and Food sciences. Butterworths, London – Boston.

Terblanche, J. H., Wooldridge, L. G., Hesebeck, I. and Joubert, M. 1979. The redistribution and immobilisation of calcium in apple trees with special reference to bitter pit. *Communications in Soil Science & Plant Analysis 10* (1-2), 195-215.

Tromp, J. 1980. Mineral absorption and distribution in young apple trees under various environmental conditions. In: *Mineral nutrition of fruit trees.* pp. 173 – 182. Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M. Studies in the Agricultural and Food Sciences. Butterworths, London – Boston.

Vamerali, T., Bandiera, M. and Mosca, G. 2012. Minirhizotrons in modern root studies. *Measuring roots*. Mancuso, S (Ed.), Springer- Verlag Berlin Heidelberg, p. 341-356.

Van der Boon, J. 1980. Dressing or spraying Ca for bitter pit control. In: *Mineral nutrition of fruit trees* pp. 309-315. Atkinson, D., Jackson, J.E., Sharples, R.O. and Waller, W.M. Studies in the Agricultural and Food Sciences. Butterworths, London – Boston.

Vang-Petersen, O. 1980. Calcium nutrition of apple trees: a review. *Scientia Horticulturae 12*(1), 1-9.

Vargas, O. L. 2015. Nitrogen fertigation practices to optimize growth and yield of Northern Highbush Blueberry (*Vaccinium corymbosum* L.). A Ph.D. dissertation submitted to Oregon State University.

White, P. J. 2001. The pathways of calcium movement to the xylem. *Journal of Experimental Botany* 52(358), 891-899.

White, P. J. and Broadley, M. R. 2003. Calcium in plants. *Annals of Botany 92*(4), 487-511.

Wiersum, L.K. 1980. The effect of soil physical conditions on roots and uptake. In: Mineral nutrition of fruit trees.pp. 111-122. Atkinson, D., Jackson, J.E., Sharples, R.O. and

Waller, W.M. Studies in the Agricultural and Food Sciences. Butterworths, London – Boston.

Wilsdorf, R.E. 2011. Evaluating the seasonal changes in calcium concentration and distribution in apple fruit after application of diffferent calcium fertilisation strategies. MSc thesis in the Department of Horticultural Sceince, Faculty of Agricultural Science, Stellenbosch University.

Zimmerman, M. H., Brown, C. L. and Tyree, M. T. 1971. Trees: Structure and Function. Springer – Verlag, New York Inc, p. 51-57.

Table 1: The corresponding dates and rates of $Ca(NO_3)_2$ applications applied in the potted trial on young, bearing 'Golden Delicious' trees according to treatment (X = 8 g.pot^{-1}).

Treatment	Rate	Application Date					
		27 Nov 2013	4 Dec 2013	26 Dec 2013	14 Mar 2014	21 Mar 2014	4 Apr 2014
Summer	1X	4g	4g	-	-	-	-
Summer high	3X	8g	8g	8g	-	-	-
Autumn	1X	-	-	-	4g	4g	-
Autumn high	3X	-	-	-	8g	8g	8g
Summer & Autumn	1X	4g	4g	-	4g	4g	-
Summer & Autumn high	3X	8g	8g	8g	8g	8g	8g

Table 2: The phenological timing and corresponding dates at which the two treatments of $Ca(NO_3)_2$ were applied in the field trial on mature, bearing 'Golden Delicious' trees at the recommended rate of 232 g.tree^{-1} except where indicated.

Treatment	Timing	Application date			
		2013		2014	
Industry	Post harvest	3 May	11 May	12 May	19 May
	90% petal drop	25 Oct	2 Dec*	7 Nov**	14 Nov**
Root flush	1st flush	10 Apr	15 Apr	31 May	6 Jun
	2nd flush	4 Dec	23 Dec	14 Nov**	5 Dec**

*Faulty timing of application
**696 g.tree^{-1}

Table 3: Calcium % in roots, shoots (new growth including leaves) and trunk tissue respectively for young 'Golden Delicious'/M7 as determined at harvest for Ca applied to pots at six different regimes versus a control where no additional Ca was supplied. Control at planting indicates the reserve Ca status of the roots and trunk. P-value determined at a 5% level.

Treatment	Roots (%)	Trunk (%)	Shoots (%)
Control at planting (avg 10 trees)	0.43	0.47	NA
Control	0.68^{c}	0.42^{ns}	0.72^{c}
Summer	0.67^{c}	0.45	0.80^{bc}
Summer high	0.74^{bc}	0.47	0.97^{ab}
Autumn	0.94^{a}	0.46	0.68^{c}
Autumn high	0.95^{a}	0.48	0.72^{c}
Summer & Autumn	0.97^{a}	0.41	0.82^{bc}
Summer & Autumn high	0.93^{ab}	0.46	1.04^{a}
P-Value	0.0048	0.3281	0.0097
LSD	0.2020	0.0688	0.2108

Means with different letters differed significantly at $P < 0.05$. Means followed by "ns" were not significantly different.

Table 4: Trunk diameter (mm) and tree height (cm) of young, non-bearing potted 'Golden Delicious' trees after soil application of $Ca(NO_3)_2$ according to six different regimes.

Treatment	Diameter	Length
Control	12.33^{ns}	159.83^{abc}
Summer	13.25	149.00^{c}
Summer high	12.58	153.33^{bc}
Autumn	13.17	151.00^{c}
Autumn high	12.75	153.17^{bc}
Summer & Autumn	12.33	165.33^{ab}
Summer & Autumn high	12.25	171.33^{a}
P-Value	0.5709	0.0226
LSD	1.3042	14.014

Means with different letters differed significantly at $P < 0.05$. Means followed by "ns" were not significantly different.

Table 5: Fresh and dry mass of the roots, trunk and shoots (new growth incl. leaves) of young, non-bearing potted 'Golden Delicious' trees after soil application of $Ca(NO_3)_2$ at six different regimes and a control where no additional Ca was supplied. P-value and LSD were determined at a 5% level. Analysis represents all 9 replications per treatment.

	Fresh mass (g)			Dry mass (g)		
Treatment	Roots	Trunk	Shoots	Roots	Trunk	Shoots
Control	520.0^{ns}	142.3^{ns}	183.4^{ns}	324.1^{ns}	79.0^{ns}	94.2^{ns}
Summer	481.3	120.3	186.9	286.2	69.0	97.9
Summer high	478.9	119.7	176.8	290.3	68.2	93.1
Autumn	358.9	113.1	185.2	202.4	64.9	92.2
Autumn high	424.6	121.6	178.1	250.7	68.4	93.7
Summer & Autumn	353.1	133.4	213.1	204.6	75.6	106.2
Summer & Autumn high	395.0	134.1	242.2	229.4	73.0	110.0
P-Value	0.0957	0.1245	0.1511	0.0658	0.1556	0.2285
LSD	133.35	22.15	53.07	90.60	10.87	16.94

Means with "ns" was not significantly different.

Table 6: Average one year-old shoot growth on 12/06/2013 and 19/05/2014 for mature 'Golden Delicious' trees.

Treatment	2013 (cm)	2014 (cm)
Control	29.19^{ns}	30.48^{ns}
Industry	28.17	32.89
Root flush	28.09	35.83
P-Value	0.8431	0.1146
LSD	4.3506	5.0806

Means with "ns" were not significantly different.

Table 7: Yields and Yield efficiency for mature 'Golden Delicious' trees for 2013/2014 and 2014/2015 seasons after two different $Ca(NO_3)_2$ application regimes.

Treatment	Total Harvest 2014 (kg)	Total Harvest 2015 (kg)	Yield efficiency 2014 (kg/cm^2)	Yield efficiency 2015 (kg/cm^2)
Control	89.46^{ns}	71.562^{ns}	1.37^{ns}	0.82^{ns}
Industry	89.66	67.18	1.40	0.85
Root flush	99.65	73.769	1.49	0.84
P-Value	0.4914	0.8511	0.6821	0.9798
LSD	19.88	24.30	0.29	0.31

Means with "ns" were not significantly different

Table 8: Fruit maturity evaluation at harvest (03/04/2014) for mature 'Golden Delicious' trees in which ten fruit per plot was used for analysis after two different $Ca(NO_3)_2$ application regimes.

Treatment	Background (green)	Starch (%)	Firmness (kg)	Diameter (mm)	Mass (g)	TSS (°Brix)
Control	2.50^{ns}	26.00^{ns}	7.50^{ns}	74.02^{ns}	161.93^{ns}	12.18^{ns}
Industry	2.48	26.06	7.51	73.71	160.78	11.96
Root flush	2.43	27.50	7.50	73.31	157.36	12.17
P-Value	0.9378	0.7468	0.9989	0.7613	0.7691	0.3646
LSD	0.39	4.57	0.22	1.98	13.49	0.36

Means with "ns" were not significantly different.

Table 9: Fruit quality evaluation after 8 weeks of storage at 13/05/2014 for mature 'Golden Delicious' trees in which 20 fruit per plot was used for analysis.

Treatment	Background (green)	Firmness (kg)	Diameter (mm)	Mass (g)	TSS (°Brix)	Sunburn
Control	2.33^{ns}	6.64^{ns}	73.80^{ns}	155.58^{ns}	13.37^{ns}	0.50^{ns}
Industry	2.44	6.76	73.60	153.01	13.38	0.44
Root flush	2.21	6.66	73.54	152.13	13.36	0.45
P-Value	0.0535	0.5770	0.9728	0.8775	0.9960	0.8039
LSD	0.18	0.24	2.34	14.42	0.51	0.20

Means with "ns" were not significantly different.

Table 10: Fruit maturity evaluation at harvest (20/02/2015) for mature 'Golden Delicious' trees in which ten fruit per plot was used for analysis after two different $Ca(NO_3)_2$ application regimes.

Treatment	Background (green)	Starch (%)	Firmness (kg)	Diameter (mm)	Mass (g)	TSS (°Brix)
Control	2.30^{ns}	21.20^{ns}	7.42^{ns}	59.76^{ns}	164.77^{ns}	13.52^{ab}
Industry	2.10	22.70	7.41	59.33	159.10	13.26^{b}
Root flush	2.20	25.50	7.38	59.58	162.50	13.94^{a}
P-Value	0.4625	0.6109	0.9681	0.4938	0.5256	0.0230
LSD	0.48	8.90	0.32	0.74	10.24	0.48

Means with different letters differed significantly at $P < 0.05$. Means with "ns" were not significantly different.

Table 11: Fruit mineral analysis for macro elements at harvest (2014) for mature 'Golden Delicious' trees in which six fruit per plot was used for analysis after two different $Ca(NO_3)_2$ application regimes.

Treatment	N	P	K	Ca	Mg	Fruit mass
	mg/100g fresh mass					(g)
Control	63.00^{ns}	7.45^{ns}	116.00^{ns}	4.82^{ns}	5.27^{ns}	140.17^{ns}
Industry	64.22	8.39	125.22	5.10	5.43	136.19
Root flush	66.89	8.33	123.00	5.08	5.32	132.72
P-Value	0.7072	0.7058	0.6754	0.9029	0.8331	0.2146
LSD	9.79	2.57	22.24	1.41	0.58	8.49

Means with "ns" were not significantly different.

Table 12: Fruit mineral analysis of for macro elements at harvest (2015) for mature 'Golden delicious' trees in which six fruit per plot was used for analysis after two different $Ca(NO_3)_2$ application regimes.

Treatment	N	P	K	Ca	Mg	Fruit mass
	mg/100g fresh mass					(g)
Control	39.33^{ns}	9.00^{ns}	132.22^{ns}	4.60^{ns}	5.52^{ns}	166.37^{ns}
Industry	41.11	8.80	134.22	4.61	5.62	164.89
Root flush	42.00	9.36	132.11	4.77	5.66	159.06
P-Value	0.8505	0.8912	0.9674	0.8603	0.9132	0.2694
LSD	9.82	2.44	19.02	0.70	0.67	9.59

Means with "ns" were not significantly different.

Table 13: Leaf mineral analysis for macro and micro mineral elements (07/02/2014) for mature, bearing 'Golden Delicious' trees.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	B (mg/kg)
Control	2.52^{ns}	0.18^{ns}	1.33^{ns}	1.62^{ns}	0.30^{ns}	150.33^{ns}	580.33^{b}	141.00^{ns}	7.33^{ns}	118.78^{b}	27.67^{ns}
Industry	2.59	0.17	1.35	1.61	0.30	164.89	672.33^{a}	172.22	7.44	153.67^{a}	27.89
Root flush	2.48	0.16	1.26	1.76	0.31	171.00	688.00^{a}	143.33	7.44	155.89^{a}	27.78
P-Value	0.2998	0.448	0.521	0.2457	0.8813	0.0632	0.0268	0.6306	0.9313	0.0084	0.971
LSD	0.15	0.03	0.17	0.20	0.06	17.59	82.60	74.04	0.70	25.07	1.89

Means with different letters differed significantly at $P < 0.05$. Means with "ns" were not significantly different.

Table 14: Leaf mineral analysis for macro and micro mineral elements (27/01/2015) for mature, bearing 'Golden Delicious' trees.

Treatment	N (%)	P (%)	K (%)	Ca (%)	Mg (%)	Na (mg/kg)	Mn (mg/kg)	Fe (mg/kg)	Cu (mg/kg)	Zn (mg/kg)	B (mg/kg)
Control	3.09[a]	0.18[ns]	1.41[ns]	1.69[ns]	0.33[ns]	181.67[ns]	794.67[ns]	115.44[ns]	8.33[ns]	228.00[ns]	33.89[ns]
Industry	2.64[b]	0.17	1.48	1.63	0.33	183.67	724.22	123.56	8.22	208.11	36.33
Root flush	3.09[a]	0.17	1.38	1.73	0.32	177.56	793.89	107.11	8.11	231.00	34.78
P-Value	0.0345	0.9573	0.4735	0.6897	0.9808	0.8366	0.2917	0.6739	0.7767	0.4035	0.1944
LSD	0.39	0.04	0.18	0.24	0.07	21.45	103.65	37.89	0.64	37.40	2.73

Means with different letters differed significantly at $P < 0.05$. Means with "ns" were not significantly different.

Table 15 a: Soil analysis for macro mineral elements in 0-30cm soil of the mature, bearing 'Golden Delicious' trees in June 2013 after two different $Ca(NO_3)_2$ application regimes.

Treatment	pH (KCl)	P (mg/kg)	K (mg/kg)	Na cmol(+)/kg)	K cmol(+)/kg)	Ca cmol(+)/kg)	Mg cmol(+)/kg)	C (%)
Control	5.62^{ns}	55.22^{ns}	226.56^{ns}	0.12^{ns}	0.58^{ns}	9.40^{ns}	2.95^{ns}	2.52^{ns}
Industry	5.56	76.22	253.67	0.11	0.65	9.15	2.98	2.54
Root flush	5.48	83.00	211.44	0.12	0.54	9.12	2.64	2.47
P-Value	0.4196	0.4176	0.1921	0.6722	0.2083	0.8097	0.7807	0.6010
LSD	0.22	44.42	46.96	0.02	0.12	0.97	1.11	0.15

Means with "ns" were not significantly different.

Table 15 b : Soil analysis for micro elements in 0-30cm soil of the mature 'Golden Delicious' trees in June 2013 after two different $Ca(NO_3)_2$ application regimes.

Treatment	Cu (mg/kg)	Zn (mg/kg)	Mn (mg/kg)	B (mg/kg)	Fe (mg/kg)
Control	7.99^{ns}	9.71^{ns}	40.51^{ns}	0.76^{ns}	113.69^{ns}
Industry	8.77	10.76	53.06	0.96	110.14
Root flush	7.60	10.47	52.29	0.86	111.85
P-Value	0.8720	0.8858	0.1499	0.3637	0.9848
LSD	4.69	4.51	14.31	0.29	41.79

Means with "ns" were not significantly different.

Table 16: Soil analysis for macro mineral elements in 0-30 cm soil of the mature, bearing 'Golden Delicious' trees in June 2014 after two different $Ca(NO_3)_2$ application regimes.

Treatment	pH (KCl)	P (Bray) (mg/kg)	K (Bray) (mg/kg)	Na cmol(+)/kg)	K cmol(+)/kg)	Ca cmol(+)/kg)	Mg cmol(+)/kg)	C (%)
Control	5.73^{ns}	51.56^{ns}	185.89^{ns}	0.14^{ns}	0.47^{ns}	8.93^{ns}	2.76^{ns}	2.51^{ns}
Industry	5.66	72.00	179.44	0.14	0.46	9.29	2.61	2.60
Root flush	5.59	75.44	172.33	0.14	0.44	8.79	2.55	2.59
P-Value	0.6195	0.4259	0.7256	0.9955	0.7363	0.6999	0.9100	0.5212
LSD	0.30	40.07	34.71	0.028	0.09	1.24	1.01	0.17

Means with "ns" were not significantly different.

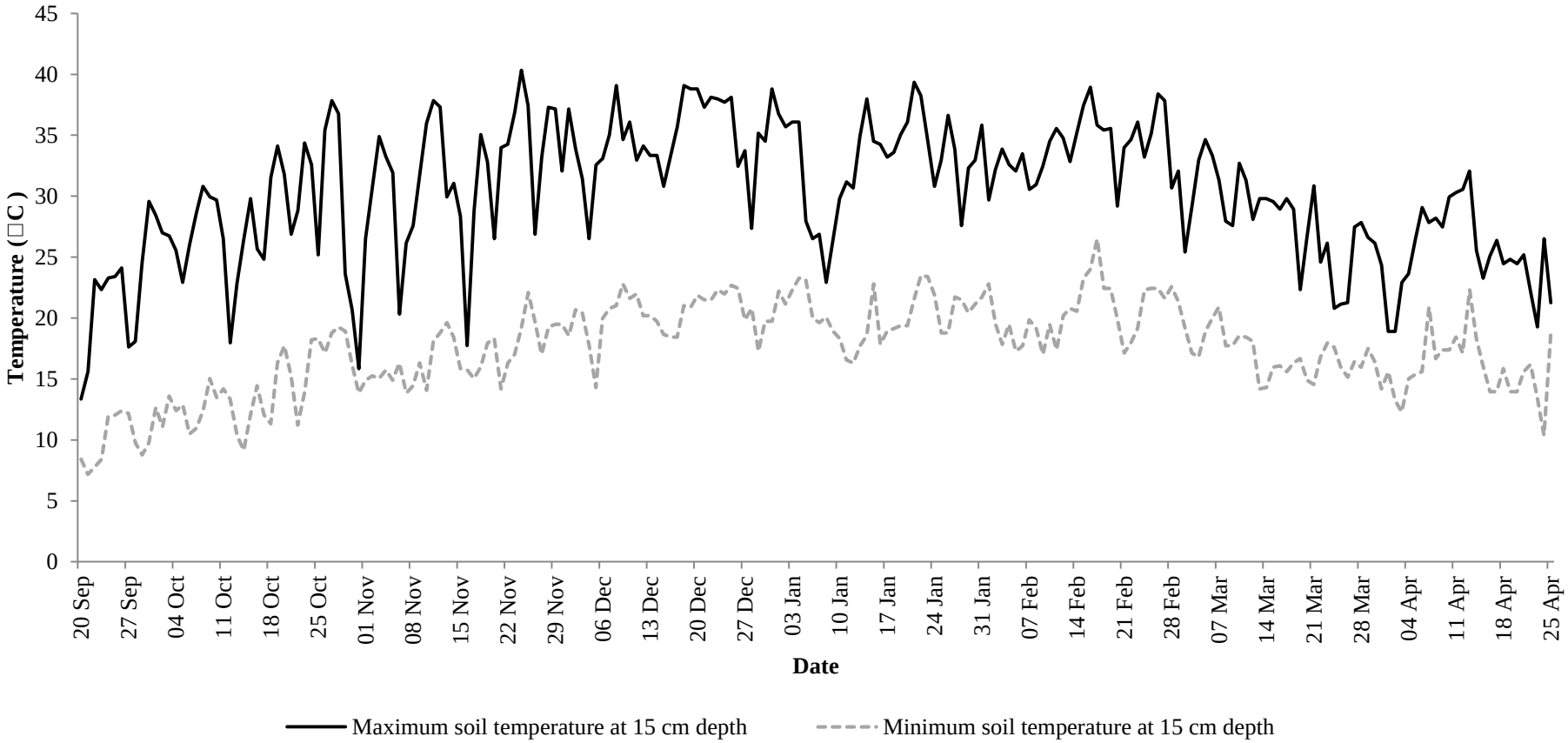

Figure 1: Temperatures (°C) of potted sand medium at 15 cm depth from just after planting (20/09/2013) to harvesting (25/04/2014) of young, non-bearing 'Golden Delicious' trees under glasshouse conditions.

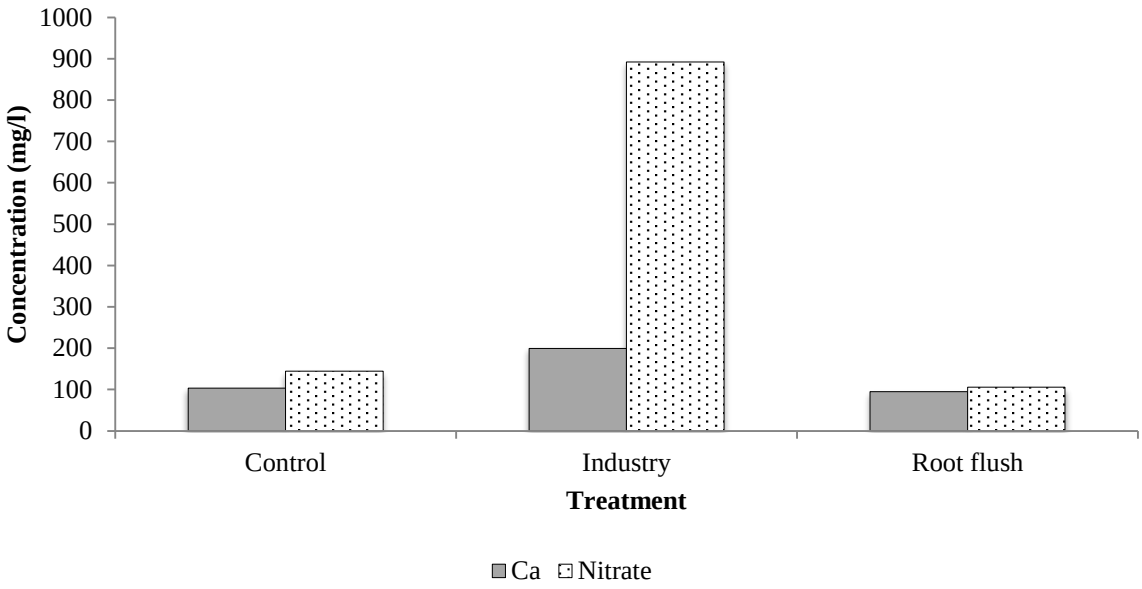

Figure 2: The average concentration (mg/l) of Ca^{2+} and NO_3^- in the soil solution extracted at a soil depth of 30 cm following the soil application of the industry treatment (232 g.tree^{-1} $Ca(NO_3)_2$) on 25 October 2013.

General Conclusion

The main objective of this study was to determine if the dynamics of white root production in the selected South African apple orchards agreed with reports from the Northern hemisphere and whether white root growth patterns differ between bearing and non-bearing trees. Potential correlations between root growth and soil temperature, soil water content and tree physiology were also investigated. This information was then applied to determine if trees and fruit would benefit from additional soil applied calcium applications if synchronized with the white root growth flushes.

The timing, duration and magnitude of white root growth flushes were quantified for four different cultivars on two contrasting soil types in the Elgin-Vyeboom region of Western cape. The four orchards were as follows: young, non-bearing 'Corder Gala'/M7 on a sandy soil (Vyeboom); young, bearing 'Fuji'/M793 on a clay loam soil (Applegarth); mature, bearing 'Golden Delicious'/M793 on a clay loam soil (Applegarth) and 'Cripps pink'/M793 on a sandy soil (Somersfontein). The timing of root growth flushes were similar for the three mature bearing orchards, irrespective of the different scions, but the duration and magnitude of the flushes differed.

The white root growth pattern of the young, non-bearing orchard clearly differed from the mature, bearing orchards indicating the significant influence of crop load on root production patterns. White root production in the mature, bearing orchards was distinctly periodic, whereas the young trees produced roots in various quantities throughout the growing season with no consistent white root growth during winter. In contrast, the mature, bearing orchards consistently produced the majority of roots during winter with another, often smaller root flush during summer. Although a summer flush is commonly reported for apple in the Northern hemisphere, it usually occurs during bloom and root growth during winter is seldom reported. In our study minimum winter soil temperatures, especially at 60 cm soil depth (5 - 12°C), are conducive to root growth. Although the soil environmental conditions throughout the year was conducive to root growth, no direct correlation was found between the dynamics of white root numbers and changes in soil temperature or water content. The dynamics of root production in our study therefore seems to be controlled by endogenous tree physiological factors. A possible relationship between white root numbers and photosynthesis for the young non-bearing trees, but it was not evident for the mature, bearing trees, again confirming the complexity of bearing

trees reported before. Further studies regarding the direct physiological correlation with root growth are needed under controlled environmental conditions to increase our understanding of carbon usage by root growth.

In addition, two separate trials were conducted where i) the effect of timing and concentration of soil applied $Ca(NO_3)_2$ on the accumulation and distribution of Ca amongst different tree parts (roots, trunk and new growth) were quantified for potted 'Golden Delicious' trees and ii) the effect of applying $Ca(NO_3)_2$ during root flushes were quantified a mature, bearing 'Golden Delicious' orchard. The pot trial showed that higher levels of soil applied Ca are required during summer for increasing Ca concentration of the new arial growth compared to application during autumn were the standard application sufficed to increase the Ca concentration of the root system. In contrast, no effect on leaf or fruit Ca concentration was detected under field conditions after applying $Ca(NO_3)_2$ to the soil during root growth flushes. The lack of effect under field conditions was mainly attributed to the recommended rate of application being too low and was confirmed by the substantially higher rates applied in other studies with positive results. Other factors which most likely contributed to the lack in uptake efficiency included suboptimal irrigation scheduling and inefficient ground cover management.

Future research should include field trial applications of sufficient rates of soil applied Ca to increase the reserve status of the trees, similar to that recorded in the potted plants. This could impact on fruit Ca status early during the following season and contribute towards reducing Ca related physiological deficiencies like bitter pit.

Another possible research opportunity would be to investigate white root dynamics in local areas with higher winter chilling, like the Koue Bokkeveld and Eastern Free State, with regards to the extent of the duration of the second root flush during winter dormancy.

Finally it would be of importance to quantify the possible effect of the extended winter root flush with regards to the utilization of the carbon reserves in the tree. It should be determined if this has any negative effect on tree growth after dormancy and if so, how to address this phenomenon on nutritional level.

Appendix

Table 1: Average values for photosynthesis (µmol CO_2 m^{-2} s^{-1}) during 2013 and 2014 according to date and T=Tube (replicate) number for bearing 'Golden Delicious'/M793 trees.

Season 1	T1	T2	T3	T4	T5	T6	T7	T8	T9
04/03/2013	17.4	18.1	18.6	20.4	20.9	19.8	14.89	13.7	16.4
11/03/2013	17.1	19.0	19.6	18.8	20.9	20.5	18.18	16.9	18.5
18/03/2013	14.5	12.4	11.7	12.5	16.3	15.0	9.94	12.1	10.8
25/03/2013	15.8	14.1	13.3	12.9	17.2	16.3	15.17	13.6	17.6
03/04/2013	14.4	12.9	13.0	13.9	14.4	15.6	13.68	10.4	16.8
10/04/2013	15.1	13.9	13.1	14.0	15.3	12.9	12.45	13.1	13.6
15/04/2013	13.9	12.0	12.9	12.8	15.5	13.1	13.2	8.0	14.7
22/04/2013	14.8	16.3	13.4	15.8	15.6	12.4	11.04	12.2	14.6
13/05/2013	15.7	14.2	15.9	15.4	12.7	8.1	11.78	16.4	14.5
Season 2	T1	T2	T3	T4	T5	T6	T7	T8	T9
18/11/2013	17.8	21.8	24.3	24.9	27.4	27.6	18.3	23.1	22.8
02/12/2013	14.8	13.9	12.8	13.4	13.2	13.2	16.4	11.9	13.3
23/12/2013	17.5	15.6	13.6	16.6	18.3	21.0	15.4	15.1	11.7
16/01/2014	14.7	20.2	15.3	16.4	16.6	16.1	16.6	17.2	15.6
07/02/2014	13.4	17.1	15.1	15.3	16.9	12.9	16.6	13.5	13.3
20/02/2014	16.6	17.7	16.4	18.7	19.3	19.3	16.0	16.8	18.0
06/03/2014	17.1	17.0	15.5	18.6	15.9	17.0	16.6	16.0	14.8
20/03/2014	12.9	14.7	13.1	15.6	14.6	13.1	12.4	12.0	13.1
03/04/2014	14.1	15.5	15.2	18.3	13.8	17.3	16.7	13.3	13.8
17/04/2014	11.6	11.9	13.1	15.1	11.5	14.0	12.0	12.2	9.9

Table 2: Average values for photosynthesis (µmol CO_2 m^{-2} s^{-1}) during 2013 and 2014 according to date and T=Tube (replicate) number for non - bearing 'Corder Gala'/M7 trees.

Season 2	T1	T2	T3	T4	T5
25/11/2013	7.5	10.3	8.4	10.0	10.1
16/12/2013	19.3	20.1	17.7	17.8	19.3
13/01/2014	12.4	17.3	18.7	12.9	15.4
30/01/2014	18.1	20.2	17.2	17.0	18.4
13/02/2014	20.9	19.0	18.2	18.1	19.0
27/02/2014	20.1	20.0	14.1	15.5	18.0
13/03/2014	17.8	12.6	14.1	13.7	16.7
27/03/2014	19.6	18.3	16.6	17.9	16.7
10/04/2014	16.8	19.1	13.1	16.6	13.5
25/04/2014	19.5	14.5	10.3	15.0	10.7

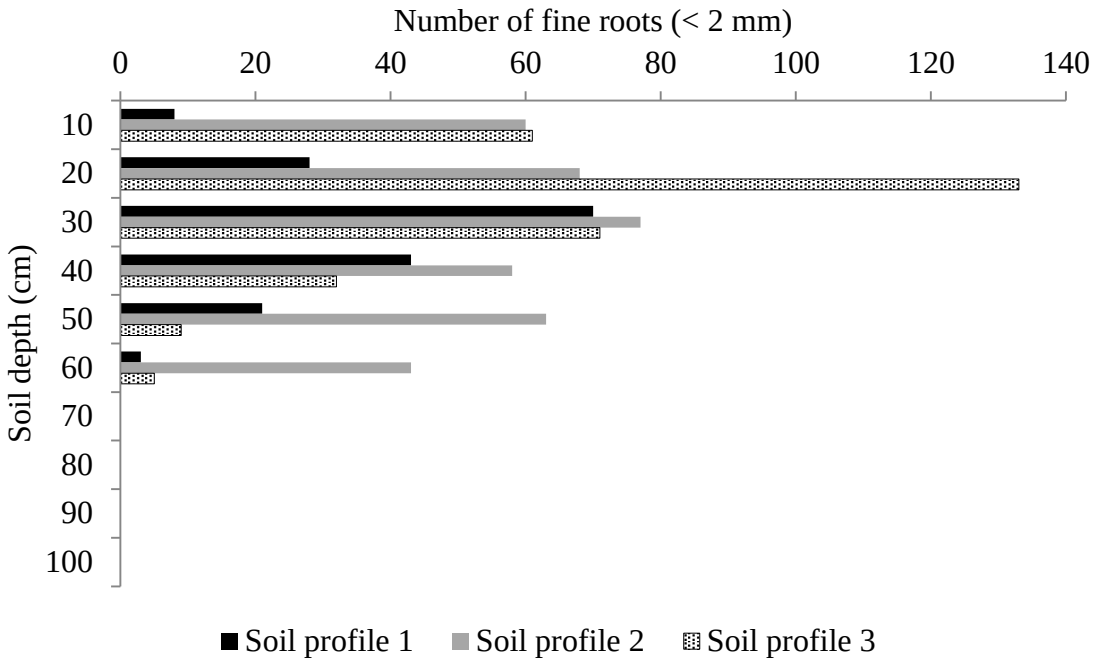

Figure 1: The number of fine (< 2mm diameter) roots according to soil depth obtained from the root distribution study of three young, non – bearing 'Corder Gala'/M7 trees on 14 May 2013.

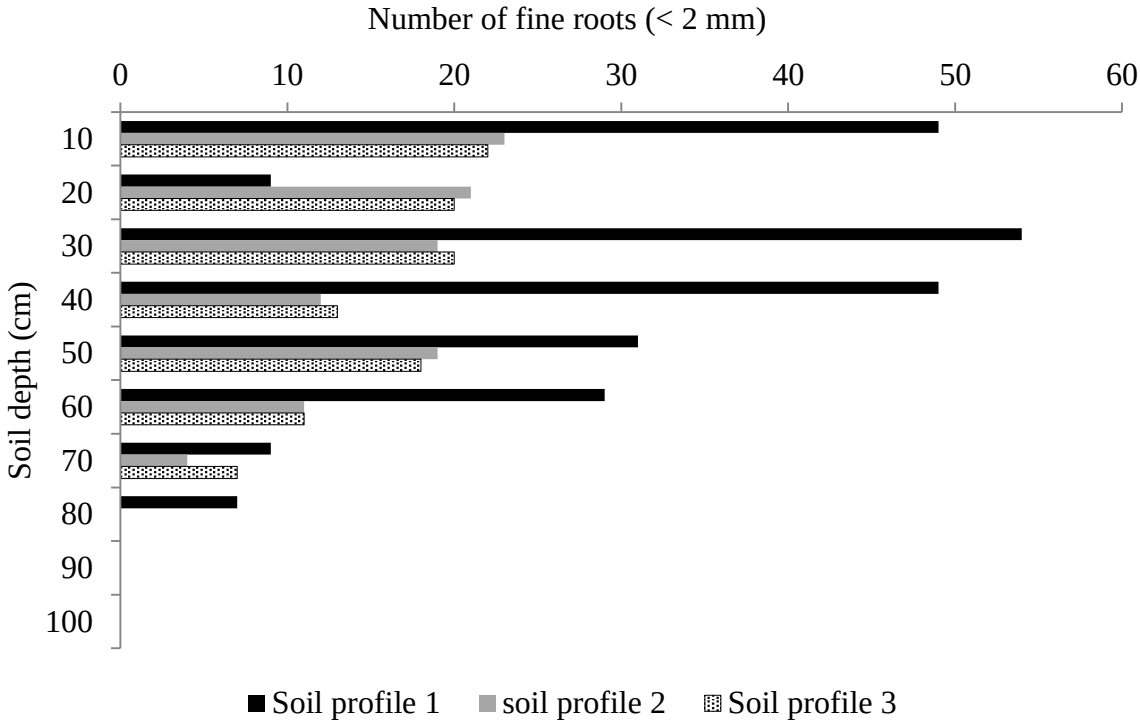

Figure 2: The number of fine (< 2mm diameter) roots according to soil depth obtained from the root distribution study of three young, non – bearing 'Corder Gala'/M7 trees on 19 May 2014.

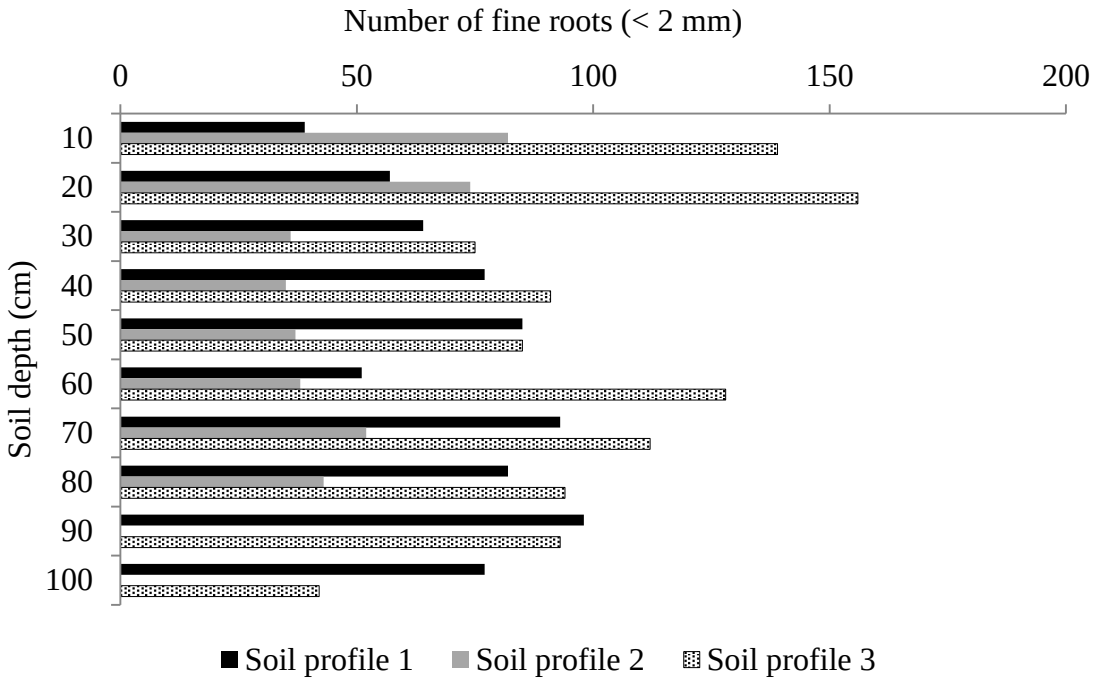

Figure 3: The number of fine (< 2mm diameter) roots according to soil depth obtained from the root distribution study of three mature, bearing 'Golden Delicious'/M793 trees on 5 June 2013.

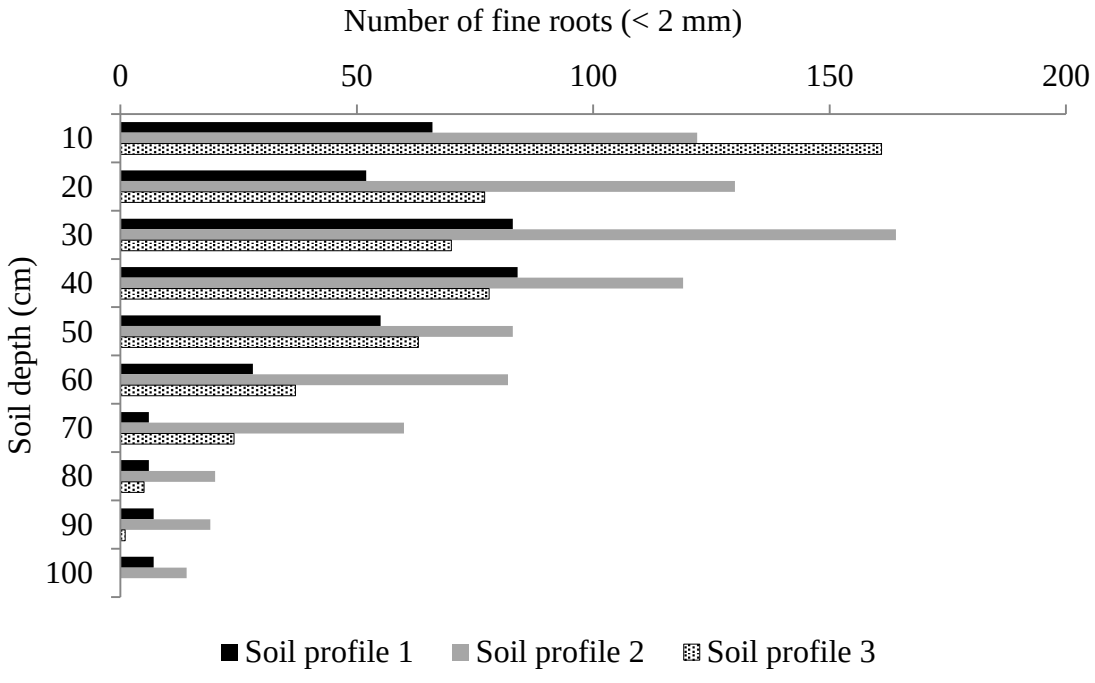

Figure 4: The number of fine (< 2mm diameter) roots according to soil depth obtained from the root distribution study of three mature, bearing 'Golden Delicious'/M793 trees on 12 May 2014.

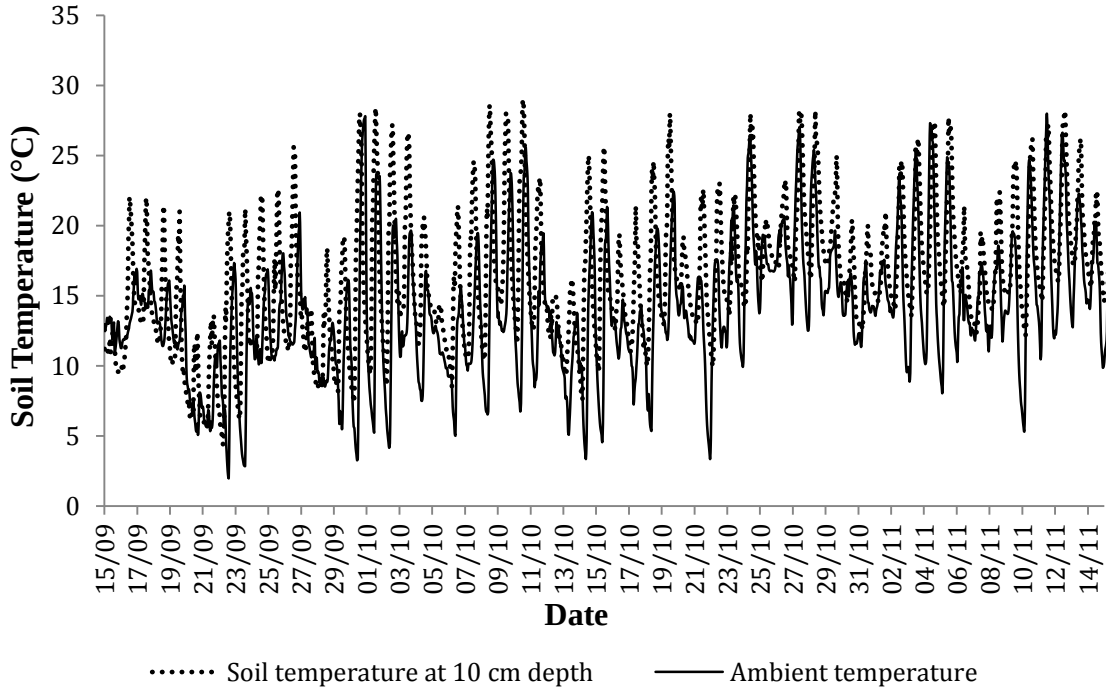

Figure 5: The hourly fluctuation in ambient and soil temperature (10 cm depth) for the mature, bearing bearing 'Golden Delicious'/M793 orchard from 15 September 2013 until 14 November 2013.

Figure 6: Status of weed and grass on 18 November 2013 at the mature, bearing 'Golden Delicious'/M793 orchard before herbicide treatment.

Figure 7: Status of weed and grass on 16 January 2014 at the mature, bearing 'Golden Delicious'/M793 orchard after herbicide treatment.